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L6: Entry 44 of 176 File: USPT Aug 4, 1998

DOCUMENT-IDENTIFIER: US 5789191 A
TITLE: Method of detecting and counting microorganisms

CLAIMS:

13. The method according to claim 1, wherein the selective medium is for testing enterococci and consists of 30 to 60 parts by weight of a customary base medium and one or more selectors selected from the group consisting of sodium citrate, sodium azide, thallium acetate and 2,3,5-triphenyltetrazole.

L30: Entry 9 of 28 File: USPT Apr 6, 1999

DOCUMENT-IDENTIFIER: US 5891499 A
TITLE: Composition for eliminating unsanitary residues from food products and method for using the same

Detailed Description Text (23):
The used culture media were triptosium agar for the total count, the McConvey's soil for E. Coli and coliforms, and the Escherichia azide agar soil for the Streptococci D.

that produced optimal specific activity (125 to 175 nmol of C2H2
reduced/min per mg of total protein). The apparent Michaelis constants
(Km)for the magnesium adenosine triphosphate complex, reducible substrates
azide , acetylene, and N2 and the nonphysiological electron donor
hydrosulfite (S2O42-) were determined to be 0.7, 0.7, 0.2, 0.06, and 0.03
MM, respectively. These apparent Km values are in reasonable agreement with
those reported for the nitrogenases of Azotobacter vinelandii and
Klebsiella pneumoniae. Either a total lack of cooperativity between binding
sites or a single binding site for reducible substrates is indicated by
analysis of Hill plots. Hill plot slopes of approximately 1.7 suggest that
multiple binding sites exist for both ATP and S2O42-.
Tags: Support, U.S. Gov't, Non-P.H.S.
Descriptors: *Bacillus--enzymology--EN; *Nitrogenase--metabolism--ME; Acetylene--metabolism--ME; Adenosine Triphosphate--metabolism--ME; Anaerobiosis ; Azides --metabolism--ME; Azotobacter--enzymology--EN; Binding Sites; Cell-Free System; Kinetics; Klebsiella pneumoniae --enzymology--EN; Nitrogen--metabolism--ME; Species Specificity; Sulfites --metabolism--ME
CAS Registry No.: 0 (Azides); 0 (Sulfites); 56-65-5 (Adenosine Triphosphate); 74-86-2 (Acetylene); 7727-37-9 (Nitrogen)
Enzyme No.: EC 1.18.6.1 (Nitrogenase)
Record Date Created: 19760706

6/9/6 (Item 6 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

12190987 BIOSIS NO.: 199900485836
Recovery of Escherichia coli Biotype I and Enterococcus spp. during
refrigerated storage of beef carcasses inoculated with a fecal slurry.
AUTHOR: Calicioglu M; Buege D R; Ingham S C; Luchansky J B(a)
AUTHOR ADDRESS: (a)Department of Food Science, and Department of Food
Microbiology and Toxicology, University of Wisconsin, Madison, Madison,
WI, 53706**USA
JOURNAL: Journal of Food Protection 62 (8):p944-947 Aug., 1999
ISSN: 0362-028X
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English
SUMMARY LANGUAGE: English

ABSTRACT: Three beef front quarters/carcasses were inoculated with a slurry
of cattle manure. During storage at 4degreeC, two sponge samples from
each of three sites (i.e., 100 cm2 from each of two fat surfaces and 100
cm2 from a lean surface) were taken from each of the three carcasses on
days 0, 1, 3, 7, and 10 after inoculation. The initial numbers of
Escherichia coli averaged 2.0 log10 CFU/cm2 (1.21 to 2.47 log10 CFU/cm2)
using the Petrifilm method and 2.09 log10 most probable number (MPN)/cm2
(0.88 to 2.96 log10 MPN/cm2) using the MPN method. The initial numbers of
enterococci averaged 3.34 log10 CFU/cm2 (3.07 to 3.79 log10 CFU/cm2)
using kanamycin esculin azide agar. In general, an appreciable
reduction in the numbers of E. coli occurred during the first 24 h of
storage; for the Petrifilm method an average reduction of 1.37 log10
CFU/cm2 (0.69 to 1.71 log10 CFU/cm2) was observed, and for the MPN method
an average reduction of 1.52 log10 MPN/cm2 (0.47 to 2.08 log10 MPN/cm2)
was observed. E. coli were not detected (<-0.12 log10 CFU/cm2) using
Petrifilm on day 7 of the storage period on two (initial counts of 1.21
and 2.29 log10 CFU/cm2) of the three carcasses. However, viable E. coli
cells were recovered from these two carcasses after a 24-h enrichment at
37degreeC in EC broth. Viable E. coli cells were detected at levels of
-0.10 log10 CFU/cm2 on the third carcass (initial count of 2.47 log10
CFU/cm2) after 7 days at 4degreeC. No significant difference in recovery
of viable cells was observed between the MPN and Petrifilm methods on
days 0, 1, and 3 ($P > 0.05$). However, viable E. coli cells were recovered
from all three carcasses by the MPN method on day 7 at an average of
-0.29 log10 MPN/cm2 (-0.6 to -0.1 log10 MPN/cm2). On day 10, viable cells
were recovered by the MPN method from two of the three carcasses at -0.63
and -0.48 log10 MPN/cm2 but were not recovered from the remaining carcass
(<-0.8 log10 MPN/cm2).Similar to E. coli, the greatest reduction (average
of 1.26 log10 CFU/cm2, range = 1.06 to 1.45 log10 CFU/cm2) in the numbers

of enterococci occurred during the first 24 h of storage. Because of
higher initial numbers and a slightly slower rate of decrease, the
numbers of Enterococcus spp. were significantly higher ($P < 0.017$) than
the numbers of E. coli Biotype I after 3, 7, and 10 days of storage.
These results suggest that enterococci may be useful as an indicator of
fecal contamination of beef carcasses.

DESCRIPTORS:
MAJOR CONCEPTS: Foods; Methods and Techniques
BIOSYSTEMATIC NAMES: Enterobacteriaceae--Facultatively Anaerobic
Gram-Negative Rods, Eubacteria, Bacteria, Microorganisms; Gram-Positive
Cocci--Eubacteria, Bacteria, Microorganisms
ORGANISMS: Enterococcus spp. (Gram-Positive Cocci)--food contaminant;
Escherichia coli (Enterobacteriaceae)--biotype I, food contaminant
BIOSYSTEMATIC CLASSIFICATION (SUPER TAXA): Bacteria; Eubacteria;
Microorganisms
METHODS & EQUIPMENT: most probable number method--food contaminant
detection method; refrigerated storage--food storage method;
Petrifilm method--food contaminant detection method
MISCELLANEOUS TERMS: beef carcasses--fecal contamination, meat
CONCEPT CODES:
39008 Food and Industrial Microbiology-General and Miscellaneous
13502 Food Technology-General; Methods
23001 Temperature: Its Measurement, Effects and Regulation-General
Measurement and Methods
BIOSYSTEMATIC CODES:
06702 Enterobacteriaceae (1992-)
07700 Gram-Positive Cocci (1992-)

6/9/2 (Item 2 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

12333320 BIOSIS NO.: 200000086822
Mechanism of anti- and pro-oxidant effect of azide on pentachlorophenol
metabolite-induced toxicity.
AUTHOR: Zhu Ben-Zhan(a); Levy Smadar(a); Chevion Mordechai(a)
AUTHOR ADDRESS: (a)Department of Cellular Biochemistry, Hebrew
University-Hadassah Medical School, Jerusalem, 91120**Israel
JOURNAL: Free Radical Biology & Medicine 27 (SUPPL. 1):pS127 1999
CONFERENCE/MEETING: 6th Annual Meeting of the Oxygen Society New Orleans,
Louisiana, USA November 18-22, 1999
SPONSOR: The Oxygen Society
ISSN: 0891-5849
RECORD TYPE: Citation
LANGUAGE: English
REGISTRY NUMBERS: 66-71-7: 1 10-PHENANTHROLINE; 14343-69-2: AZIDE ; 7440-50-8: COPPER; 70-51-9: DESFERRIOXAMINE; 1198-55-6:
TETRACHLOROCATECHOL
DESCRIPTORS:
MAJOR CONCEPTS: Metabolism; Toxicology
BIOSYSTEMATIC NAMES: Enterobacteriaceae--Facultatively Anaerobic
Gram-Negative Rods, Eubacteria, Bacteria, Microorganisms
ORGANISMS: Escherichia coli (Enterobacteriaceae)--model system
BIOSYSTEMATIC CLASSIFICATION (SUPER TAXA): Bacteria; Eubacteria;
Microorganisms
CHEMICALS & BIOCHEMICALS: 1,10-phenanthroline; azide --preservative,
toxin; copper; desferrioxamine; tetrachlorocatechol--preservative,
toxin
METHODS & EQUIPMENT: ESR--analytical method, spectroscopic techniques--CB
, spectroscopic techniques--CT; UV/visible studies--radiobiology
method
MISCELLANEOUS TERMS: cytotoxicity; wood; Meeting Abstract
CONCEPT CODES:
22501 Toxicology-General; Methods and Experimental
06502 Radiation-General
31000 Physiology and Biochemistry of Bacteria
00520 General Biology-Symposia, Transactions and Proceedings of
Conferences, Congresses, Review Annuals
BIOSYSTEMATIC CODES:
06702 Enterobacteriaceae (1992-)

6/9/21 (Item 21 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

11827656 BIOSIS NO.: 199900073765
The inherent genotoxic potency of food mutagens and other heterocyclic and
carbocyclic aromatic amines and corresponding azides .
AUTHOR: Wild D(a); Kerdar R S
AUTHOR ADDRESS: (a)Fed. Cent. Meat Res., Inst. Microbiol. Toxicol.,
E.-C.-Baumann-Str. 20, D-95326 Kulmbach**Germany
JOURNAL: Zeitschrift fuer Lebensmittel-Untersuchung und -Forschung A 207 (
6):p427-433 1998

ISSN: 1431-4630
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Relationships between the chemical structure of aromatic amines
(including heterocyclic food mutagens) and genotoxic potency were
originally established on the basis of Salmonella mutagenicity data.
These relationships are reviewed. We report here that also quite
different genotoxic effects, namely the binding to
deoxyguanosine-3'-phosphate (dGp), hypoxanthine
phosphoribosyl-transferase (HPRT) mutations, and sister chromatid
exchange in Chinese hamster cells follow essentially the same
structure-activity relationships. The heterocyclic amines of the
aminoimidazoquinoline, aminoimidazoquinoxaline and aminoimidazopyridine
types unite a number of structural characteristics which endow these
compounds, or rather their reactive species, presumed to be nitrenium
ions, with an extremely high inherent genotoxic potency. This conclusion
is supported by experimental work and by calculations of electronic
properties of these compounds and their nitrenium ions.

REGISTRY NUMBERS: 14343-69-2D: AZIDES
DESCRIPTORS:
MAJOR CONCEPTS: Foods; Molecular Genetics (Biochemistry and Molecular
Biophysics); Toxicology
BIOSYSTEMATIC NAMES: Cricetidae--Rodentia, Mammalia, Vertebrata, Chordata
, Animalia; Enterobacteriaceae-- Facultatively Anaerobic
Gram-Negative Rods, Eubacteria, Bacteria, Microorganisms
ORGANISMS: Salmonella typhimurium (Enterobacteriaceae); V79 cell line
(Cricetidae)--Chinese hamster fibroblasts
BIOSYSTEMATIC CLASSIFICATION (SUPER TAXA): Animals; Bacteria; Chordates;
Eubacteria; Mammals; Microorganisms; Nonhuman Mammals; Nonhuman
Vertebrates; Rodents; Vertebrates
CHEMICALS & BIOCHEMICALS: azides --food residue, quantitative
structure-activity relationships, genotoxicity; carbocyclic aromatic
amines--food residue, genotoxicity, quantitative structure-activity
relationships; heterocyclic aromatic amines--food residue,
quantitative structure-activity relationships, genotoxicity
METHODS & EQUIPMENT: hypoxanthine ribosyltransferase assay--analytical
method; sister chromatid exchange test--assessment method; Ames test
--assessment method
MISCELLANEOUS TERMS: food products--cooked
CONCEPT CODES:
13502 Food Technology-General; Methods
02506 Cytology and Cytochemistry-Animal
03502 Genetics and Cytogenetics-General
10060 Biochemical Studies-General
10502 Biophysics-General Biophysical Studies
22501 Toxicology-General; Methods and Experimental
BIOSYSTEMATIC CODES:
06702 Enterobacteriaceae (1992-)
86310 Cricetidae

6/9/16 (Item 16 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

11940555 BIOSIS NO.: 199900186664
Active efflux and diffusion are involved in transport of Pseudomonas·
aeruginosa cell-to-cell signals.
AUTHOR: Pearson James P; van Delden Christian; Iglewski Barbara H(a)
AUTHOR ADDRESS: (a)Department of Microbiology and Immunology, University of
Rochester, 601 Elmwood Ave., Rochester,**USA
JOURNAL: Journal of Bacteriology 181 (4):p1203-1210 Feb., 1999
ISSN: 0021-9193
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Many gram-negative bacteria communicate by N-acyl homoserine
lactone signals called autoinducers (AIs). In Pseudomonas aeruginosa,
cell-to-cell signaling controls expression of extracellular virulence
factors, the type II secretion apparatus, a stationary-phase sigma factor
(sigmas), and biofilm differentiation. The fact that a similar signal,
N-(3-oxohexanoyl) homoserine lactone, freely diffuses through Vibrio
fischeri and Escherichia coli cells has led to the assumption that all
AIs are freely diffusible. In this work, transport of the two P.
aeruginosa AIs, N-(3-oxododecanoyl) homoserine lactone (3OC12-HSL)
(formerly called PAI-1) and N-butyryl homoserine lactone (C4-HSL)
(formerly called PAI-2), was studied by using tritium-labeled signals.
When (3H)C4-HSL was added to cell suspensions of P. aeruginosa, the
cellular concentration reached a steady state in less than 30 s and was

nearly equal to the external concentration, as expected for a freely
diffusible compound. In contrast, (3H)3OC12-HSL required about 5 min to
reach a steady state, and the cellular concentration was 3 times higher
than the external level. Addition of inhibitors of the cytoplasmic
membrane proton gradient, such as azide , led to a strong increase in
cellular accumulation of (3H)3OC12-HSL, suggesting the involvement of
active efflux. A defined mutant lacking the mexA-mexB-oprM-encoded
active-efflux pump accumulated (3H)3OC12-HSL to levels similar to those
in the azide -treated wild-type cells. Efflux experiments confirmed
these observations. Our results show that in contrast to the case for
C4-HSL, P. aeruginosa cells are not freely permeable to 3OC12-HSL.
Instead, the mexA-mexB-oprM-encoded efflux pump is involved in active
efflux of 3OC12-HSL. Apparently the length and/or degree of substitution
of the N-acyl side chain determines whether an AI is freely diffusible or
is subject to active efflux by P. aeruginosa.

REGISTRY NUMBERS: 672-15-1QD: HOMOSERINE; 1927-25-9QD: HOMOSERINE
DESCRIPTORS:
MAJOR CONCEPTS: Biochemistry and Molecular Biophysics; Chemical
Coordination and Homeostasis; Infection
BIOSYSTEMATIC NAMES: Bacteria--Microorganisms; Enterobacteriaceae--
Facultatively Anaerobic Gram-Negative Rods, Eubacteria, Bacteria,
Microorganisms; Pseudomonadaceae--Gram-Negative Aerobic Rods and Cocci,
Eubacteria, Bacteria, Microorganisms
ORGANISMS: gram-negative bacteria (Bacteria); Escherichia coli
(Enterobacteriaceae)--pathogen; Pseudomonas aeruginosa
(Pseudomonadaceae)--pathogen
ORGANISMS: PARTS ETC: secretion apparatus
BIOSYSTEMATIC CLASSIFICATION (SUPER TAXA): Bacteria; Eubacteria;
Microorganisms
CHEMICALS & BIOCHEMICALS: sigma factors; N-acyl homoserine lactone
MISCELLANEOUS TERMS: cell-to-cell signalling; efflux pumps; virulence
factors
CONCEPT CODES:
31000 Physiology and Biochemistry of Bacteria
10060 Biochemical Studies-General
12002 Physiology, General and Miscellaneous-General
12502 Pathology, General and Miscellaneous-General
36002 Medical and Clinical Microbiology-Bacteriology
13002 Metabolism-General Metabolism; Metabolic Pathways
30500 Morphology and Cytology of Bacteria
BIOSYSTEMATIC CODES:
05000 Bacteria-General Unspecified (1992-)
06508 Pseudomonadaceae (1992-)
06702 Enterobacteriaceae (1992-)

6/9/14 (Item 14 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

11948709 BIOSIS NO.: 199900194818
Role of the lateral channel in catalase HPII of Escherichia coli.
AUTHOR: Sevinc M Serdal; Mate Maria J; Switala Jack; Fita Ignacio; Loewen
Peter C(a)
AUTHOR ADDRESS: (a)Department of Microbiology, University of Manitoba,
Winnipeg, MB, R3T 2N2**Canada
JOURNAL: Protein Science 8 (3):p490-498 March, 1999
ISSN: 0961-8368
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The heme-containing catalase HPII of Escherichia coli consists of
a homotetramer in which each subunit contains a core region with the
highly conserved catalase tertiary structure, to which are appended N-
and C-terminal extensions making it the largest known catalase. HPII does
not bind NADPH, a cofactor often found in catalases. In HPII, residues
585-590 of the C-terminal extension protrude into the pocket
corresponding to the NADPH binding site in the bovine liver catalase.
Despite this difference, residues that define the NADPH pocket in the
bovine enzyme appear to be well preserved in HPII. Only two residues that
interact ionically with NADPH in the bovine enzyme (Asp212 and His304)
differ in HPII (Glu270 and Glu362), but their mutation to the bovine
sequence did not promote nucleotide binding. The active-site heme groups
are deeply buried inside the molecular structure requiring the movement
of substrate and products through long channels. One potential channel is
about 30 ANG in length, approaches the heme active site laterally, and is
structurally related to the branched channel associated with the NADPH

binding pocket in catalases that bind the dinucleotide. In HPII, the
upper branch of this channel is interrupted by the presence of Arg260
ionically bound to Glu270. When Arg260 is replaced by alanine, there is a
threefold increase in the catalytic activity of the enzyme. Inhibitors of
HPII, including azide , cyanide, various sulfhydryl reagents, and
alkylhydroxylamine derivatives, are effective at lower concentration on
the Ala260 mutant enzyme compared to the wild-type enzyme. The crystal
structure of the Ala260 mutant variant of HPII, determined at 2.3 ANG
resolution, revealed a number of local structural changes resulting in
the opening of a second branch in the lateral channel, which appears to
be used by inhibitors for access to the active site, either as an inlet
channel for substrate or an exhaust channel for reaction products.

REGISTRY NUMBERS: 9001-05-2: CATALASE; 14875-96-8: HEME; 9001-05-2: EC
1.11.1.6; 58-68-4: NADH
DESCRIPTORS:
MAJOR CONCEPTS: Enzymology (Biochemistry and Molecular Biophysics)
BIOSYSTEMATIC NAMES: Animalia; Bacteria--Microorganisms;
Enterobacteriaceae-- Facultatively Anaerobic Gram-Negative Rods,
Eubacteria, Bacteria, Microorganisms; Fungi--Plantae
ORGANISMS: animals (Animalia); bacteria (Bacteria); yeasts (Fungi);
Escherichia coli (Enterobacteriaceae)
BIOSYSTEMATIC CLASSIFICATION (SUPER TAXA): Animals; Bacteria; Eubacteria;
Fungi; Microorganisms; Nonvascular Plants; Plants
CHEMICALS & BIOCHEMICALS: catalase HPII {EC 1.11.1.6}--analysis,
heme-containing enzyme, lateral channel roles, molecular
characteristics; heme; oligonucleotides; reaction products--analysis
; solvents; NADH
METHODS & EQUIPMENT: enzyme purification--Isolation/Purification
Techniques--CB, purification method; mutagenesis--molecular genetic
method, molecular genetics/genetic engineering,
oligonucleotide-directed; PCR-Mate synthesizer--Applied Biosystems,
equipment; X-ray crystallography--X-ray analysis, analytical method
CONCEPT CODES:
31000 Physiology and Biochemistry of Bacteria
10050 Biochemical Methods-General
10060 Biochemical Studies-General
10502 Biophysics-General Biophysical Studies
10506 Biophysics-Molecular Properties and Macromolecules
10802 Enzymes-General and Comparative Studies; Coenzymes
10806 Enzymes-Chemical and Physical
BIOSYSTEMATIC CODES:
05000 Bacteria-General Unspecified (1992-)
06702 Enterobacteriaceae (1992-)
15000 Fungi-Unspecified
33000 Animalia-Unspecified

6/9/15 (Item 15 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

11941209 BIOSIS NO.: 199900187318
Conditional stability of the HemA protein (glutamyl-tRNA reductase)
regulates heme biosynthesis in Salmonella typhimurium.
AUTHOR: Wang Liying; Elliott Meenal; Elliott Thomas(a)
AUTHOR ADDRESS: (a)Department of Microbiology and Immunology, WVU Health
Sciences Center, Morgantown, WV, 26506-917**USA
JOURNAL: Journal of Bacteriology 181 (4):p1211-1219 Feb., 1999
ISSN: 0021-9193
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: In many bacteria, including the enteric species Salmonella
typhimurium and Escherichia coli, heme is synthesized starting from
glutamate by a pathway in which the first committed step is catalyzed by
the hemA gene product, glutamyl-tRNA reductase (HemA). We have
demonstrated previously that when heme limitation is imposed on cultures
of S. typhimurium, HemA enzyme activity is increased 10- to 25-fold.
Western (immunoblot) analysis with monoclonal antibodies reactive with
HemA revealed that heme limitation results in a corresponding increase in
the abundance of the enzyme. Similar regulation was also observed for E.
coli. The near absence of regulation of hemA-lac operon fusions suggested
a posttranscriptional control. We report here the results of
pulse-labeling and immunoprecipitation studies of this regulation. The
principal mechanism that contributes to elevated HemA abundance is
protein stabilization. The half-life of HemA protein is simeq20 min in
unrestricted cells but increases to >300 min in heme-limited cells.

Similar regulation was observed for a HemA-LacZ hybrid protein containing
almost all of the HemA protein (416 residues). Sodium azide prevents
HemA turnover in vivo, suggesting a role for energy-dependent
proteolysis. This was confirmed by the finding that HemA turnover is
completely blocked in a lon clpP double mutant of E. coli. Each single
mutant shows only a small effect. The ClpA chaperone, but not ClpX, is
required for ClpP-dependent HemA turnover. A hybrid HemA-LacZ protein
containing just 18 amino acids from HemA is also stabilized in the lon
clpP double mutant, but this shorter fusion protein is not correctly
regulated by heme limitation. We suggest that the 18 N-terminal amino
acids of HemA may constitute a degradation tag, whose function is
conditional and modified by the remainder of the protein in a
heme-dependent way. Several models are discussed to explain why the
turnover of HemA is promoted by Lon-ClpAP proteolysis only when
sufficient heme is available.
REGISTRY NUMBERS: 119940-26-0: GLUTAMYL-TRNA REDUCTASE; 14875-96-8: HEME; 9037-80-3: REDUCTASE
DESCRIPTORS:
MAJOR CONCEPTS: Enzymology (Biochemistry and Molecular Biophysics); Metabolism
BIOSYSTEMATIC NAMES: Bacteria--Microorganisms; Enterobacteriaceae-- Facultatively Anaerobic Gram-Negative Rods, Eubacteria, Bacteria, Microorganisms
ORGANISMS: bacteria (Bacteria); Escherichia coli (Enterobacteriaceae); Salmonella typhimurium (Enterobacteriaceae)
BIOSYSTEMATIC CLASSIFICATION (SUPER TAXA): Bacteria; Eubacteria; Microorganisms
CHEMICALS & BIOCHEMICALS: amino acids; enzymes; glutamyl-transfer RNA reductase--conditional stability, molecular characteristics, functions; heme--biosynthesis regulation; molecular chaperones; HemA protein-- conditional stability, molecular characteristics, functions
METHODS & EQUIPMENT: Western blotting--analytical method, detection/labeling techniques
MISCELLANEOUS TERMS: proteolysis
CONCEPT CODES:
31000 Physiology and Biochemistry of Bacteria
10060 Biochemical Studies-General
10802 Enzymes-General and Comparative Studies; Coenzymes
31500 Genetics of Bacteria and Viruses
36002 Medical and Clinical Microbiology-Bacteriology
32000 Microbiological Apparatus, Methods and Media
13002 Metabolism-General Metabolism; Metabolic Pathways
30500 Morphology and Cytology of Bacteria
BIOSYSTEMATIC CODES:
05000 Bacteria-General Unspecified (1992-)
06702 Enterobacteriaceae (1992-)

6/9/63 (Item 3 from file: 144)
DIALOG(R)File 144:Pascal
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05845412 PASCAL No.: 84-0346860
Temperature-dependent azide sensitivity of growth and ATPase activity in the facultative thermophile, Bacillus coagulans
JONES M V; SPENCER W N; EDWARDS C
Univ. Liverpool, dep. microbiology, Liverpool L69 3BX, United Kingdom
Journal: Journal of general Microbiology, 1984, 130 (1) 95-101
ISSN: 0022-1287 Availability: CNRS-4410
No. of Refs.: 24 ref.
Document Type: P (Serial) ; A (Analytic)
Country of Publication: United Kingdom
Language: English
L'inhibition de la croissance de Bacillus coagulans par l'aide de sodium decroit quand la temperature de croissance augmente alors que le contenu en cytochrome et particulierement en cytochrome augmente. L'activite de l'ATPase est sensible a l' azide mais l'inhibition varie a la fois avec la croissance et la temperature

English Descriptors: Bacillus coagulans; Inhibition; Growth; Temperature; Enzyme; ATPase; Enzymatic activity; Cytochrome; Anaerobiosis ; Sensitivity resistance; Metabolism; Bacteria
French Descriptors: Bacillus coagulans; Inhibition; Croissance; Temperature ; Enzyme; ATPase; Activite enzymatique; Cytochrome; Anaerobiose ; Sensibilite resistance; Metabolisme; Bacterie; Sodium Azoture

Classification Codes: 002A05B13

6/9/64 (Item 1 from file: 149)
DIALOG(R)File 149:TGG Health&Wellness DB(SM)
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01086285 SUPPLIER NUMBER: 03904127 (THIS IS

THE FULL TEXT)
Geomicrobiology of hydrothermal vents.
Jannasch, Holger W.; Mottl, Michael J.
Science, v229, p717(9)
Aug 23,
1985
PUBLICATION FORMAT: Magazine/Journal ISSN: 0036-8075 LANGUAGE: English
RECORD TYPE: Fulltext TARGET AUDIENCE: Academic
WORD COUNT: 5516 LINE COUNT: 00537

TEXT:

Deep-sea hydrothermal vents were discovered in the 1970's after an extensive search along the Galapagos Rift (1, 2), a part of the globe-encircling system of sea-floor spreading axes. During the past 7 years, more hydrothermal vent fields have been located along the East Pacific Rise. They fall into two main groups: (i) warm vent fields with maximum exit temperatures of 5 degrees to 23 degrees C and flow rates of 0.5 to 2 cm sec.sup.-1 and (ii) hot vent fields with maximum exit temperatures of 270 degrees to 380 degrees C and flow rates of 1 to 2 m sec.sup.-1. Hot vent fields commonly include warm- and intermediate-temperature vents ([is less than or =]300 degrees C) ("white smokers") as well as high-temperature vents (350 degrees [plus-or-minus] 2 degrees C) ("black smokers"). A highly efficient microbial utilization of geothermal energy is apparent at these sites--rich animal populations were found to be clustered around these vents in the virtual absence of a photosynthetic food source (3-5).

Microorganisms, mainly bacteria, are efficient geochemical agents. As prokaryotic organisms, they lack a membrane-bound nucleus and thereby the complex genetic apparatus of the higher, eukaryotic organisms. At the same time, bacteria retain a much wider metabolic diversity than is found in plants and animals. Because of the resulting biochemical versatility of natural microbial populations and the smallness, general resistance, and dispersibility of bacterial cells, these organisms are able to exist in more extreme environments than the higher organisms. Therefore, the occurrence of certain microorganisms at deep-sea vents was predictable; however, their ability to make it possible for higher forms of life to thrive with an unusual efficiency on inorganic sources of energy in the absence of light was entirely unexpected.

Chemosynthesis

The most significant microbial process taking place at the deep-sea vents is "bacterial chemosynthesis." The term was coined by Pfeffer in 1897 (6) in obvious contrast to the then well-known photosynthesis. Both processes involve the biosynthesis of organic carbon compounds from CO.sub.2., with the source of energy being eiher chemical oxidations or light, respectively. More specifically, chemoautotrophy refers to the assimilation of CO.sub.2. and is coupled in some bacteria to chemolithotrophy, the ability to use certain reduced inorganic compounds as energy sources.

In the present-day terminology, the relation between photosynthetic and chemosynthetic metabolism is illustrated in the following schematic equations, where the reduced carbon is represented as a carbohydrate, [CH.sub.2.O]:

From an evolutionary point of view, reactions 1 and 2 above are bridged by the blue-green or cyanobacteria. In aerobic chemosynthesis, the possible electron donors used by a large variety of bacteria are listed in Table 1. Some of them are the same as those used in anaerobic chemosynthesis where free oxygen is replaced by NO.sub.3-, elemental sulfur, SO.sub.4.sup.2-, or CO.sub.2 as electron acceptors. The inorganic sources of energy are used for the production of ATP (adenosine 5'-triphosphate), akin to the use of light in phototrophy.

Differences in the average growth rates of chemolithotrophic bacteria under comparable conditions are determined by the amount of energy required for "reverse electron transfer," a metabolic mechanism required for generating the necessary negative redox potential. Some organisms have the ability to use organic compounds simultaneously as electron sources (mixotrophy). Since in autotrophy carbon (CO.sup.2) must be reduced from a higher oxidative state than organic carbon, more energy is required than in heterotrophy. Therefore, obligate chemoautrophic bacteria generally grow more slowly than heterotrophs or require larger amounts of substrate in terms of energy supply.

During recent years a number of new types of anaerobic

chemoautotrophic bacteria have been isolated and described. Among them are methanogens, acetogens, and sulfate-reducing bacteria (7). In addition, it has been shown that certain extremely thermophilic methanogens are able to respire elemental sulfur (8, 9). All these metabolic types are potential catalysts of geochemical transformations at deep-sea vents.

All the inorganic energy sources listed in Table 1 have been found in hydrothermal fluids or in waters surrounding the vents except thiosulfate, the occurrence of which has not been specifically studied. Before discussing those types of bacteria that have been isolated and those microbial processes that have been shown to occur, we will outline the hydrothermal origin and the documented occurrence of the critical inorganic species.

Sources of H.sub.2.S and Structure of the Mixing Region

The chemistry of the vent waters indicates that both warm and hot vent fields are fed at depth by a high-temperature end-member solution at about 350 degrees C and that the mixing of this solution with largely unreacted and unheated ocean bottom water in the shallow regions of the crust is responsible for the wide range of exit temperatures (2, 10). Thus, chemical species that are nonreactive during mixing define mixing lines as a function of temperature for the warm vent waters. These lines pass through ambient seawater and extrapolate to a composition at 350 degrees C similar to that actually measured in the hot vent waters.

Most of the chemical species thought to participate in microbiological reactions do not exhibit such linear mixing behavior. These species may therefore originate either at depth in the high-temperature end-member solution that has been produced by reaction of heated seawater with crustal rocks (11), or they may originate in the shallow subsea-floor region, either directly from bottom sea-water or as a result of various inorganic and organic reactions that occur on mixing (Fig. 1).

The concentrations of relevant species from the best-studied hot vent fields (those on the East Pacific Rise near 21 degrees N) and warm vent fields (those on the Galapagos Rift near 86 degrees W) are shown in Table 2. Also shown are the results of two model calculations, the first for a conservative mixture of the hot vent waters with ocean bottom water and the second for the same mixture after some simplified inorganic reactions have occurred.

The prominence of H.sub.2.S is obvious from Table 2. There are two possible sources for H.sub.2.S in the hot vent waters: it may be leached from crustal basalts, or it may be produced by reduction of SO.sub.4/.sup.2- from seawater coupled with oxidation of Fe.sup.2+ from basalt to Fe.sup.3+. Both mechanisms are important in laboratory experiments at 300 degrees C and above, but they occur only sluggishly or not at all at lower temperatures (12). It is likely that both mechanisms are important in the natural system as well. The concentration of sulfur in typical mid-ocean ridge basalt ([is approxi.]25 mmol/kg as S.sup.2-) is similar to that in seawater ([is approxi.]28 mmol/kg as SO.sub.4.sup.2-), and seawater circulating through the hydrothermal system of a mid-ocean ridge apparently reacts with an amount of fresh rock about equal to its own mass (10, 13, 14). Although the hot vent waters are essentially free of SO.sub.4.sup.2-, circulating seawater can be expected to lose some or all of its load of SO.sub.4.sup.2- as anhydrite (CaSO.sub.4), which precipitates on heating to temperatures as low as 130 degrees C (15). Thus, little seawater SO.sub.4.sup.2- may be delivered to the deeper, hotter parts of the system where it could be reduced to S.sup.2-. Sulfur isotopic analyses of H.sub.2.S from the hot vent waters and of sulfide minerals from the precipitated vent chimneys indicate that H.sub.2.S is derived mainly from the basalts, but that the seawater source also is important (16).

The conservatively calculated H.sub.2.S concentration in Table 2 for a 12.6 degrees C mixture of hot vent water with seawater is in the same range as those in the warm vent waters at the same temperature. H.sub.2.S undoubtedly is not conservative during subsurface mixing, however, as Fe.sup.2+, O.sub.2, and NO.sub.3- are all heavily depleted in the warm vent waters, presumably as a result of reaction with H.sub.2.S. Examination of the relation between vent temperature and the concentrations of species that react on mixing in the shallow subsea-floor provides insight into the

structure of the shallow crustal mixing region and the chemical processes
that occur there. This mixing region, with its large area of basalt
surfaces, which serve as substrate, and its dual source of electron donors
from the hot water end-member and electron acceptors from seawater, is a
major site of microbial production.

the generalized relation is shown in Fig. 2. For a given vent field,
O.sub.2 and NO.sub.3- decrease linearly from their values in ocean bottom
water to zero at characteristic temperatures <20 degrees C that vary from
one vent field to another (Table 3). H.sub.2.S decreases linearly with
decreasing temperature as O.sub.2 and NO.sub.3- increase, generally going
to zero at the bottom-water temperature of 2 degrees C. An inflection
typically occurs in the H.sub.2.S-temperature relation where O.sub.2 goes
to zero, with the slope of the H.sub.2.S temperature curve becoming steeper
at higher temperatures. Other species whose concentrations decrease from
their seawater values and extrapolate to zero at temperatures [is less than
or =]20 degrees to 30 degrees C in the warm vent waters are chromium,
uranium, nickel, copper, cadmium, and selenium (10).

Thus distinct zones exist in the shallow subsea-floor mixing region
that are characterized by particular redox conditions; in some cases the
boundaries between these zones are abrupt and isothermal (Fig. 2). Edmond
et al. (10) have inferred that a shallow subsurface reservoir at 10 degrees
to 32 degrees C is located beneath the warm vent fields and is being tapped
by the vents. The temperature range of this reservoir is defined by the
lowest temperatures at which specific chemical processes occur. The
inferred processes are listed in Table 3. The minimum temperatures at which
sulfide deposition occurs in the subsea-floor reservoir are those at which
nickel, copper, cadmium, and selenium go to zero; at lower temperatures,
these species are apparently unreactive and thus define mixing lines with
ambient seawater.

The NO.sub.3- concentration extrapolates to zero at a temperature
just slightly lower than the sulfide-related elements and slightly above
the highest temperature sampled; thus the reservoir is free of NO.sub.3-
and is anoxic because of the reaction of these species from seawater with
H.sub.2.S and other reduced species from the hot-water end-member. the
O.sub.2 concentration goes to zero at a temperature 1 degrees to 11 degrees
C lower than NO.sub.3-, depending on the vent field (Table 3).

These observations are best explained in terms of two distinct zones
that are shallower than the reservoir itself, in which the residence time
of the mixed waters is short relative to the rate of reduction of NO.sub.3-
or NO.sub.2- and O.sub.2, respectively. The warmer zone probably consists
of the channels that connect the reservoir to the sea floor, in which
O.sub.2 is reduced completely but NO.sub.3- is largely nonreactive. Both
NO.sub.3- and H.sub.2.S coexist in this zone (Fig. 2), which was frequently
sampled directly. The cooler zone probably consists of the throats of the
vents themselves, in which the residence time is so short that all species,
mix conservatively; H.sub.2.S, O.sub.2 and NO.sub.3- coexist in this zone.

Samples from several vents within a single vent field define single
mixing lines for reactive species. This implies that the temperatures that
bound the various zones are uniform across the area of an individual vent
field. Variation in these characteristic temperatures from one field to
another (Table 3) may reflect to some extent the variations in composition
of the hot-water end-member feeding the various fields. Probably, however,
this variation is mainly a function of the shallow crustal channel geometry
and the distribution of permeability and recharge rates of seawater to the
subsea-floor reservoir. The uniformity of the characteristic temperatures
for different vents within a single vent field reinforces the notion of a
subsurface reservoir created by permeability variations in the shallow
subsea-floor.

Because the inferred reservoir is anoxic, like the water in the
surficial upflow channels, aerobic chemosynthetic microorganisms probably
thrive mainly at the margins of these zones, where downwelling oxygenated
seawater mixes with the major bodies of already mixed and reacted
solutions. Electron-donor species from the reservoir would be available at

these sites.

Sources of Other Chemical Species Used in Chemosynthesis

In addition to H.sub.2.S, the subsea-floor reservoir contains H.sub.2 (17), although in much lower concentrations than would be expected from the high values in the hot-water end-member (Table 2). When seawater was reacted with basalts in laboratory experiments (18), the resultant concentration of H.sub.2 was lower than that in the natural 350 degrees C solutions. It apparently was controlled by the redox state, which was near the magnetite-hematite boundary at 350 degrees to 375 degrees C. The H.sub.2-O.sub.2 redox couple approached equilibrium faster than any other redox couple. Isotopic data on H.sub.2 from the hot vent waters also suggest a close approach to equilibrium for H.sub.2-H.sub.2.O (12). Inorganic reaction of H.sub.2 with O.sub.2 from seawater to relatively low temperatures during mixing could easily account for the relatively low H.sub.2 concentrations in the warm vent waters, which may then have been affected by bacterial reactions.

In contrast to H.sub.2, CH.sub.4 and CO are present at much higher concentrations in the warm vent waters than would be expected from the concentrations in the hot vent water (Table 2). CH.sub.4 in the hot vents is almost certainly abiogenic, on the basis of its similar concentration in fresh basalts and its relatively heavy isotopic composition (19), although interpretation of the isotopic data has been questioned (17). No isotopic data are available for CH.sub.4 or CO from the warm vents, but the anomalously high concentrations of these two species could well indicate a primarily biological origin, probably in the anoxic subsea-floor reservoir. As with NO.sub.3-, CH.sub.4 behaves linearly with temperature over the entire interval sampled (17), indicating that, unlike H.sub.2.S and O.sub.2, it is conserved in the inferred channels to the sea-floor. In at least one warm vent field (Rose Garden), CO apparently is produced in the upflow channels, as indicated by its inflection point and slope when plotted against temperature.

The reservoir also contains Fe.sup.2+ and Mn.sup.2+ in substantial concentrations, derived by leaching from basalt at high temperature. Mn.sup.2+ plots linearly against temperature over the entire interval sampled for the warm vents (10), and these lines extrapolate to concentrations similar to those in the 350 degrees C end-member (Table 2). Thus, Mn.sup.2+ is largely nonreactive in the shallow subsea-floor. Fe.sup.2+, by contrast, is nonlinear over the sampled interval in the same sense as H.sub.2.S (Fig. 2); thus it is being removed from solution in the upflow channels as well as in the reservoir, probably by a combination of sulfide and oxide deposition. It is uncertain to what extent Fe.sup.2+ is utilized in microbiological reactions, as it readily participates in inorganic reactions under these conditions.

Other electron donors present in the subsea-floor reservoir do not originate mainly from the hot-water end-member. NH.sub.4+ and NO.sub.2- were at or below detection limits in the 350 degrees C solutions but were readily measurable in the warm vent waters (Table 2). They almost certainly derive from reduction of seawater NO.sub.3- introduced into the reservoir, by reaction mainly with H.sub.2S. Also present at very low concentrations is N.sub.2.O (17). These species together account for less than 20 percent of the introduced NO.sub.3-; most of the rest is presumably reduced to N.sub.2. NH.sub.4+ and NO.sub.- behave linearly versus temperature over the entire interval sampled for some warm vent fields (for example, Clambake); for others, however (NO.sub.2- in Oyster Beds), they display inflection points indicating their display inflection points indicating their consumption in the upflow channels. Thiosulfate has not been sought, but elemental sulfur has been detected in warm vent effluent as well as in the chimneys of black smokers and white smokers. The slopes of plots of H.sub.2.S versus temperature for those warm vent samples that are free of O.sub.2 suggest that sulfur species with intermediate oxidation states are being formed on mixing as well as SO.sub.4.sup.2-, although SO.sub.4.sup.2- is usually dominant. Seawater also contributes SO.sub.4.sup.2- directly to the subsea-floor reservoir.

Among the electron acceptors, CO.sub.2 is paramount. This species is

highly enriched in the hot vent water by the leaching of CO.sub.2 from basalt (19, 20). Its concentration in the warm vent waters is about what it should be if the behavior of CO.sub.2 on mixing is conservative (Table 2).

Microbial Populations of Emitted Vent Waters

Without considering their specific catalytic function, one can assess abundance of natural bacterial populations by determining cell concentrations or by measuring growth rates using unspecific tracers. The milky-bluish waters (Fig. 3A) flowing from some of the warm vents (6 degrees to 23 degrees C, 1 to 2 cm sec.sup.-1) contain between 10.sup.5 and 10.sup.9 cells per milliliter (2, 4, 5). Independent of the temperatures measured, the large range of numbers is due to the dilution of vent water at the point of sampling. Visible bacterial aggregates add to this heterogeneity and may represent dislodged pieces of microbial mats (4, 5).

When contamination by ambient water was strictly prevented, we were unable to find significant numbers of microscopically visible bacteria in hot (338 degrees to 350 degrees C) vent water. In contrast, 4.7 X 10.sup.5 cells were counted in vent water at 304 degrees C (21) when the temperature was determined from magnesium concentrations (22). This finding indicated an unspecified amount of seawater intrusion prior to or during sampling.

Since aerobic chemosynthesis results in higher productivity than anaerobic chemosynthesis, the availability of the electron donor and oxygen under favorable growth conditions will be decisive. From this point of view, bacterial productivity should be highest in the vicinity of warm vents where the slow emission of sources of reduced chemical energy into oxygenated seawater forms slowly moving plumes. In contrast, the forceful emission of hydrothermal fluid from the hot vents results in a quick dispersal and fast dilution of energy sources in the water column, eventually leading to chemical oxidations. The observation of maximum populations of animals in the immediate vicinity of warm vent plumes and heavy bacterial mats near warm leakages at the base of hot vent chimneys supports these assumptions.

Biomas measurements can also be based on determinations of adenosine triphosphate (ATP) or total adenylates (22). Data of Karl et al. (5) demonstrate that the microbial biomass of warm vent plumes, determined as ATP, was two to three times that of the photosynthetic-heterotrophic microbial populations of surface waters at the same site (Galapagos Rift). The ratio of guanosine 5'-triphosphate to ATP, also measured in this study (5), has been interpreted as an indicator of growth rates. It correlated well with the data derived from biomass determinations (5).

The most recent developments in the measurement of growth rates of natural microbial populations are based on the use of tritiated nucleotides (adenine or thymidine) for incorporation into RNA and DNA (23). It is assumed that the assimilation of these marker substrates does not affect growth by stimulating ATP production. In a recent study with samples collected from a hot smoker orifice, higher adenine incorporation rates were found at 90 degrees C than at 21 degrees and 50 degrees C (24).

In addition to their occurence in warm vent water plumes (Fig. 3A), large microbial populations are also found (i) as mats covering almost indiscriminately all surfaces exposed to warm vent plumes (Fig. 3, B and C) and (ii) in symbiotic tissues within certain vent invertebrates (see below). Quantitative data on microbial activities at these two sites have not yet been obtained.

Sulfur-Oxidizing Bacteria and Rates of Chemosynthesis

The predominant chemosynthetically usable chemical energy at the vents appears in the form of sulfur compounds. This predominance is reflected in the ease and success with which sulfur-oxidizing bacteria can be isolated (25). In general, the types of sulfur bacteria found at the deep-sea vents do not differ greatly from those isolated from other H.sub.S-rich environments. There is one exception to this rule: the common occurrence of the genus Thiobacillus appears to be replaced by a prevalence of the genus Thiomicrospira (25).

Pure-culture isolations resulted in a wide range of metabolic types of sulfur bacteria including acidophilic obligate chemoautotrophs, mixotrophs (which simultaneously assimilate inorganic and organic carbon),

and facultative chemoautotrophs (25). Since the presence of organic carbon can be expected to be widespread within the vent communities, the facultative chemoautotrophs may well represent the predominant type of sulfur bacteria. The demonstrated excretion of organic carbon by obligate chemoautotrophs indicates the possible occurrence of these bacteria even in the subsurface vent systems (25). The preference for a neutral pH range favors the faculative (polythionate-producing) chemoautotrophs in the well-buffered seawater environment (26). This biochemical versatility of sulfur bacteria, together with the relatively high concentrations of reduced sulfur compounds, appears to be the key to their predominance at the vents and to their role as primary chemosynthetic producers compared to the other types of chemolithoautotrophic bacteria.

As in the measurement of photosynthesis, CO.sub.2 was used as a substrate to determine rates of chemosynthesis. With the aid of the research submersible Alvin, arrays of six 200-ml syringes were filled in situ from a joint inlet (27). They facilitated replica and control samplings and were used for in situ incubation experiments (Fig. 4). At the base of the 21 degrees N black smoker, the in situ rate of CO.sub.2 incorporation by natural microbial populations in warm water leakages was approximately 10.sup.-6 [mu]M ml.sup.-1 day.sup.-1 (27). When parallel samples were incubated in the ship's laboratory (atmospheric pressure) at 3 degrees C, the rate was virtually the same (indicating a minimal effect of hydrostatic pressure). This result was corroborated by data on the metabolic rates of a pure culture culture isolate (Thiomicrospira, strain L-12) as affected by pressure (24).

In a second shipboard incubation at 23 degrees C, the in situ temperature of the warm-water leakages, the rate of CO.sub.2 incorporation increased one and a half orders of magnitude (27). This behavior indicates the "mesophilic" growth characteristic of the total natural population. A similar response was found in pure cultures. An addition of 1mM thiosulfate as an accessory energy source in all three experiments resulted in subtantial rate increases. This immediate use of reduced sulfur confirmed the predominance of sulfur-oxidizing bacteria in the natural population (27).

Different types of dense bacterial mats have been observed at various vents (28). The genera Thiothrix and Beggiatoa appear to be predominant according to morphological criteria. During preparations for the isolation of these organisms, the capacities of marine Beggiatoa for the fixation of N.sub.2 and for facultative chemolithoautotrophy have been demonstrated (29). Whitish microbial mats and streamers were commonly observed at the base of hot vents. They represent sites of substantial chemosynthetic production and active grazing by a variety of invertebrates.

Thick mats of Beggiatoa-like filaments, partly floating above the bottom, were observed in situ at exploratory dives at the Guaymas Basin vent site (2000 m deep) in the Gulf of California (30). Collected and fixed specimens showed a filament width of up to 100 [mu]m. At this site, hot vents are overlayed by about 200 m of sediments. A substantial input of photosynthetically produced organic matter from the water column to the sediments further distinguishes this site from all others studied so far.

High concentrations of NHsub3 ([is approx.]4 mM) have also been reported (31), suggesting chemosynthesis by nitrification. A major geochemical-biological study of this site is planned for mid-1985.

Microbial CH.sub.4 Oxidation

Next to reduced sulfur, CH,sub.4, may be a substantial source of energy for chemosynthesis at those deep sea vents where it has been reported to be present in considerable quantities. Although quantitatively less abundant than H.sub.2 in the high-temperature vents, CH.sub.4 is more abundant in the warm vents (Table 2). Evidence for its microbial oxidation is, at this time, stronger than that for H.sub.2 oxidation.

Methanotrophic bacteria are included in the disparate group of the methylotrophic microorganisms, which comprise all those metabolic types that metabolize C.sub.1 compounds (32). CH.sub.4 may serve as the source of both energy and carbon (2CH.sub.4 + 2O.sub.2 right arrow 2[CH.Sub.2.O] + 2H.sub.2O), but CO.Sub.2 may be incorporated as well. All methanotrops are strictly aerobic, often microaerophilic (33), Gram-negative rods, cocci, or

vibrios and are characterized by typical intracellular membrane structures.

Methane-utilizing bacteria may also co-oxidize the CO that may occur in vent water (17), without gaining energy in the form of cell carbon through enzymes that normally catalyze other processes (34).

Microbial CH.sub.4 oxidation at the vents was first suggested when the typical morphological characteristics were observed in transmission electron micrographs from bacterial mats (Fig. 3C) (28). Up to 20 percent of the cells surveyed in sections of mats collected from various parts of the vents showed the paired vesicular membranes that distinguish methanotropic cells from similar structures found in ammonium oxidizers.

Both CH.sub.4- and methylamine-oxidizing bacteria were successfully isolated from microbial mats, Filtered vent water, clam gill tissue, And Riftia trophosome (see below), and the pure cultures obtained were preliminary grouped as type I methanotrophs (33).

Hydrogen as a Microbial Source of Energy

Many different types of microorganisms oxidize H.sub.2, but only a few are able to use the energy gained for the fixation of CO.sub.2 and can be described as chemolithoautotrophs (Table 1). Within this group the term "H.Sub.2 bacteria" is used only for aerobic organisms. Formerly grouped in the genus Hydrogenomonas, the aerobic H.sub.2-oxidizing bacteria are spread over many known genera (35). All of them are facultative autotrophs. As such, they possess ecological advantages similar to those for the facultatively autotrophic sulfur-oxidizing bacteria. They combine the properties of heterotrophic growth with the use of the Calvin cycle enzymes. The net equation for autotrophic growth is

6H.sub.2 + 2O.Sub.2 CO.sub.2 right arrow [CH.sub.2O] 5H.Sub.2O.

Little is known about the ecology of aerobic hydrogen bacteria except that their occurrence in nature is as widespread as that of biological H.sub.2-producing processes. As in the case of sulfide oxidizers, the chemosynthetic use of geothermally produced H2 at the vents represents a primary production of organic carbon. No specific study of aerobic hydrogen bacteria at the vents has yet been undertaken. An organism with a strong growth stimulation by H.sub.2 was isolated incidentally from a Riftia trophosome sample (36).

Anaerobic hydrogen-oxidizing bacteria are known as methanogens and acetogens because of their products (Table 1). They are commonly found at anoxic niches where CO.Sub.2 and H.sub.2 are present as the result of fermentation. In hydrothermal fluid both compounds are produced geothermally. The production of CH.sub.4, H.sub.2, and CO was observed experimentally at about 100[deg.]C in certain media inoculated with samples of black smoker water (37).

An extremely thermophilic methanogen of the genus Methanococcus was isolated from the base of the 21[deg.]N black smoker (Fig. 4) (38). This organism showed an optional growth rate of 0.036 hour.sup.-1 (a doubling time of 28 minutes) at 86[deg.]C. These results demonstrate the existence of a potential biological CH.Sub.4 production at the vents. The absence of isotopic evidence in support of this observation is not necessarily conclusive because of microbial patchiness.

Although denitrifying H.sub.2 oxidizers may exist in vent systems wherever the NO.sub.3./.sup.- -containing bottom seawater mixes with rising hydrothermal fluid, the SO.sub.4./.sup.2- - and sulfur-reducing equivalents are geochemically more significant. Both metabolic types of bacteria do exist but have not yet been isolated from vent waters. The respiration of elemental sulfur has recently been demonstrated to be a common property of extremely thermophilic methanogens and other archaebacteria (8, 9). Above temperatures of [is approx.]80[deg.]C, this microbial sulfur respiration occurs in addition to an abiological reduction.

Microbial Iron and Manganese Oxidation

Deposits of iron and manganese oxides cover most surfaces exposed intermittently to plumes of hydrothermal and bottom seawater or to mixes of the two. The color of these encrustations ranges from almost black to light brown. Scanning electron microscopy reveals dense microbial mats. A large variety of microbial forms are deeply embedded in the metal oxide deposits (Fig. 3, B and C).

Not enough data exist to permit estimates of the rate of mat formation. However, when various types of materials (glass,

plexiglass,
steel, membrane filters, and clam shells) enclosed in a protective rack
were placed into the opening of an active warm (21[deg.]C) vent for [is
approx.]10 months, all surfaces were evenly blackened (28, 30).

Nondispersive x-ray spectroscopy showed a decrease of the
Fe.sup.2+./.Mn.sup.2+ ratio in these layers with increasing distance from
vent openings (28), And observation attributable to the different
solubility products of the two metals. X-ray diffraction determinations of
the deposits resulted in a correlation with the mineral todorokite, (Mn,
Fe, Mg, Ca, K, Na.sub.2) . (Mn.sub.5O.sub.12) . 3H.Sub.2O, which, in its
fine-grained and poorly crystalline state, is characteristic of marine
ferromanganese deposits.

The role of bacteria in the oxidative deposition of iron is difficult to prove in neutral or alkaline waters where Fe.sup.2+ undergoes
rapid spontaneous oxidation in contact with dissolved oxygen. Heterotrophically growth bacteria have been shown to accumulate Fe.sup.3+
deposits, but no physiological significance of this process has ever been
demonstrated in the marine environment.

Although iron lithotrophy has been demonstrated for acid freshwaters
and soils true manganese lithotrophy has not been proven (39). The
oxidation of Mn.sup.2+ in seawater (pH[is approx.]8.1) is more likely than
the biological oxidation of Fe.sup.2+. Two bacterial isolates from the
Galapagos Rift vent region oxidized Mn.sup.2+ wither in growing cultures or
in cell extracts (39). The oxidation was heat-labile and inhibited by
azide (NaN3), potassium cyanide (KCNe, and antimycin A. The "oxydase" was
inducible by reduced manganese and was not constitutive as in isolates
obtained from manganese nodules. Since ATP synthesis was coupled with
Mn.sup.2+ oxidation it appears that Mn.sup.2+ - oxidizing bacteria to
contribute to the chemosynthetic production at deep-sea hydrothermal vents.

The Role of Elevated Temperatures

The transfer of thermal to chemical energy takes place at temperatures above 350[deg.]C (Fig. 1). Thermophilic CO.sub.2-,
SO.Sub.4./.sup.2- -, and S.sup.0 -reducing bacteria that use H.sub.2 as the
source of electrons (Table 1) are the best candidates for possible
microbial activities in hot zones where bottom seawater mixes below the
surface with rising hydrothermal fluid. Microbial growth has been measured
so far at temperatures up to 110[deg.]C in cultures of extremely
thermophilic bacteria isolated from shallow and deep marine hot vents (40).

The free O.Sub.2 in this mix of hydrothermal fluid and bottom
seawater may be quickly consumed biologically as well as chemically, and
both aerobic and anaerobic microorganisms may exist in subsurface vent
systems. Most aerobic bacterial isolates obtained from the turbid water
emitted by some of the Galapagos Rift warm vents were "mesophilic," that
is, exhibited growth optima at temperatures of 25[deg.] to 35[deg.]C (24).
"Extremely thermophilic" isolates obtained from the various types of
shallow and deep hot vents are all anaerobic with growth ranges from
65[deg.] to 110[deg.] and growth optima from 86[deg.] to 105[deg.]C (40).
Most of these isolates belong to the "archaebacteria," which are
distinguished from the "eubacteria" and from all eukaryotic organisms by
their specific ribosomal RNA nucleotide sequences (41).

A heterotrophic bacterium that grows on a complex organic medium
(peptone and yeast extract) in a temperature range from 55[deg.] to
98[deg.]C with an optimum at [is approx.]88[deg.]C has recently been
isolated from a shallow marine hot spring as well as from deep-sea vents
(40. It has the facultative respiration of elemental sulfur and some
other characteristics in common with the methanogenic archaebacteria (19).
The methanogenic vent isolate discussed above (38) differs from all other
archaebacteria in having a unique macrocyclic glycerol diether instead of a
tetraether as the polar membrane lipid (42), which is suspected of
affecting the membrane fluidity at high temperatures.

Bacterial growth at temperatures up to 250[deg.] by a natural
population collected from a hot vent has also been reported, but the
experimental proof of this study is still being contested (21). Other
studies with natural populations collected from the immediate vicinity of
hot ents resulted in the microbial production of gases at 100[deg.]C (37)
and in the incorporation of adenine into RNA and DNA at

rates that were higher at 90[deg.]C than at 21[deg.] and 50[deg.] (24). It has also been spectulated that the particular conditions of deep-sea hydrothermal vents might lead to a synthesis of organic compounds and ultimately to the origin of life (43).

Thorough analysis of particulate organic carbon has only been done at considerable distances from warm vent emissions (44). The results demonstrated a rather quick passage and complete transformation of microbially produced organic compounds into those characteristic of certain grazers (zooplankton). Concern about bacterial growth at hot vents is not so much a question of whethr there is a substantial addition to primary production but rather the question of the problem of biological activity at an upper temperature limit per se.

In the early spring of 1984, dense communities of marine invertebrates were also discovered at a depth of 3200 m at the base of the West Florida Escarpment, a site without volcanic or geothermic activity (45). In this area H.sub.2S-containing ground water with a salinity about one-third higher than that of the ambient seawater seeps from jointed limestone formations. The types of animals found here are similar to those described from the vent sites of the East Pacific Rise, but the individuals as well as the total quantities are smaller. The presense of H.sub.S has not been measured, but it is inferred from the odor of the collected samples. The temperatures of these nongeothermal seepages are near ambient, that is, about 0.15[deg.]C above ambient when measured at a depth of 10 cm in the sediment.

From the distributing pattern of invertebrates at the tectonic vent sites, it appears that the spotty occurrence of elevated temperature is of secondary importance for the abundance of these populations. The overriding factors seem to be the availability of inorganic chemical species and the efficiency of their use in chemosynthesis.

Symbiotic Chemosynthesis

One major evolutionary development is responsible for the unusual amounts of biomass found at the deep-sea vents: a new type of symbiosis is not commonly a topic of geomicrobiology, but this newly discovered highly efficient transformation of geothermal or geochemical energy for the production of organic carbon poses a new situation.

The predominant part of the biomass observed at the warm deep-sea vents is generated by the symbiotic association of prokarvotic cells in the clam Calpytogena magnifica and the pogonophoran tube worm Riftia pachyptila (46) (Fig. 4). The microbial symbionts have not yet been isolated, but their prokaryotic nature, DNA base ratio, genome size, and enzymatic activities identify them as bacteria (36, 47). They are found within the gill cells of C. magnifica and, as a separate "trophosome" tissue, within the body cavity of R. pachyptila. The trophosome may amount to 60 percent of the worm's wet weight.

The animal's dependence on the microbial symbiont has developed to the point where all ingestive and digestive morphological features have been lost. Through an active blood system the animal provides the bacteria in the trophosome with H.sub.2S and free O.sub.2. It appears that the spontaneous reaction of the two dissolved gases is prevented or slowed by the presence of an HS.sup.- -binding protein (48). The isolation of CH.sub.4.-oxidizing bacteria from Calyptogena gill tissue and Riftia trophosome (33) indicates, but certainly not conclusively, that chemosynthesis by CH.sub.4 assimilation (ribulose monophosphate pathway) may also take place. Enzymes associated with both the ATP-producing system and the Calvin cycle have been found in Riftia and Calyptogena.

Physiological work on purified preparations of symbionts from Riftia and the newly described vent mussel Bathymodiolus thermophilus (49) showed that their chemoautotrophic activities differ greatly with respect to temperature and the type of electron donor used (50).

Probably because of heavy predation of dying vent communities, fossilized animal remains in metal-rich deposits of ancient sea-floor spreading centers and presently mined ophiolites have only rarely been found (51). Evidence for microbial activities at similar sites has been based on the results of sulfur isotope analyses (52).

The most significant geomicrobiological point of the deep-sea vent discovery is the dependence of entire ecosystems on geothermal (terrestrial) rather than solar energy. Were a catastrophic

darkening of
the earth's surface to occur (53), the chance of survival of such
ecosystems is the highest of any community in the biosphere. The
chemosynthetic existence of organisms in the deep sea also suggests a
possible occurence of similar life forms in other planetary settings where
water may be present only in the absence of light. It is surprising that,
as far as we know, science fiction writers did not turn their attention to
geochemically supported complex forms of life until such forms were
actually discovered in the deep sea.
CAPTIONS: Electron sources and types of chemolithotropic bacteria
potentially occurring at hydrothermal vents. (table); Schematic diagram
showing inorganic chemical processes occurring at warm- and hot-water vent
sites. (chart); Comparison of the compositions of actual warm vent water at
several vent fields . (table); Tenperatures at which the concentration of
various species in seawater decrease to zero in warm vent fields on the
Galapagos Rift near 86 degrees W. (table); Relation between temperature and
the concentrations of oxygen, nitrous oxide, abd hydrogen sulfide defined
by samples from individual vents in a single warm vent field. (graph)

SPECIAL FEATURES: illustration; table; chart; photograph; graph
DESCRIPTORS: Microbiological research; Hydrothermal deposits--Research;
Galapagos Rift--Environmental aspects
FILE SEGMENT: MI File 47

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DIALOG(R)File 155:MEDLINE(R)

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Role of catalase in in vitro acetaldehyde formation by human colonic
contents.
Tillonen J; Kaihovaara P; Jousimies-Somer H; Heine R; Salaspuro M
Research Unit of Alcohol Diseases, University Central Hospital of
Helsinki, Finland.
Alcoholism, clinical and experimental research (UNITED STATES) Aug 1998
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Ingested ethanol is transported to the colon via blood circulation, and
intracolonic ethanol levels are equal to those of the blood ethanol levels.
In the large intestine, ethanol is oxidized by colonic bacteria, and this
can lead to extraordinarily high acetaldehyde levels that might be
responsible, in part, for ethanol-associated carcinogenicity and
gastrointestinal symptoms. It is believed that bacterial acetaldehyde
formation is mediated via microbial alcohol dehydrogenases (ADHs). However,
almost all cytochrome-containing aerobic and facultative anaerobic
bacteria possess catalase activity, and catalase can, in the presence of
hydrogen peroxide (H2O2), use several alcohols (e.g., ethanol) as
substrates and convert them to their corresponding aldehydes. In this study
we demonstrate acetaldehyde production from ethanol in vitro by colonic
contents in a reaction catalyzed by both bacterial ADH and catalase. The
amount of acetaldehyde produced by the human colonic contents was
proportional to the ethanol concentration, the amount of colonic contents,
and the length of incubation time, even in the absence of added
nicotinamide adenine dinucleotide or H2O2. The catalase inhibitors sodium
azide and 3-amino-1,2,4-triazole (3-AT) markedly reduced the amount of
acetaldehyde produced from 22 mM ethanol in a concentration dependent
manner compared with the control samples (0.1 mM sodium azide to 73% and
10 mM 3-AT to 67% of control). H2O2 generating system [beta-D(+)-glucose +
glucose oxidase] and nicotinamide adenine dinucleotide induced acetaldehyde
formation up to 6- and 5-fold, respectively, and together these increased
acetaldehyde formation up to 11-fold. The mean supernatant catalase
activity was 0.53+/-0.1 micromol/min/mg protein after the addition of 10 mM
H2O2, and there was a significant ($p < 0.05$) correlation between catalase
activity and acetaldehyde production after the addition of the hydrogen
peroxide generating system. Our results demonstrate that

colonic contents
possess catalase activity, which probably is of bacterial origin, and
indicate that in addition to ADH, part of the acetaldehyde produced in the
large intestine during ethanol metabolism can be catalase dependent.
Tags: Female; Human; Male; Support, Non-U.S. Gov't
Descriptors: *Acetaldehyde--pharmacokinetics--PK; *Bacteria--enzymology
--EN; *Catalase--physiology--PH; *Colon--microbiology--MI; *Digestive Tract
Contents--microbiology--MI;
*Ethanol--pharmacokinetics--PK; Adult; Aged;
Alcohol Dehydrogenase--physiology--PH; Hydrogen Peroxide--metabolism--ME;
Middle Age
CAS Registry No.: 64-17-5 (Ethanol); 75-07-0 (Acetaldehyde);
7722-84-1 (Hydrogen Peroxide)
Enzyme No.: EC 1.1.1.1 (Alcohol Dehydrogenase); EC 1.11.1.6 (Catalase)
Record Date Created: 19981215

6/9/60 (Item 1 from file: 98)
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03795794 H.W. WILSON RECORD NUMBER: BGSI98045794 (THIS IS THE FULLTEXT)
Multiple-drug resistant enterococci: the nature of the problem and an
agenda for the future.
Huycke, Mark M
Sahm, Daniel F; Gilmore, Michael S
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ABSTRACT: Enterococci, leading causes of nosocomial bacteremia, surgical
wound infection, and urinary tract infection, are becoming resistant to
many and sometimes all standard therapies. New rapid surveillance methods
are highlighting the importance of examining enterococcal isolates at the
species level. Most enterococcal infections are caused by Enterococcus
faecalis, which are more likely to express traits related to overt virulence but--for the moment--also more likely to retain sensitivity to at
least one effective antibiotic. The remaining infections are mostly caused
by E. faecium, a species virtually devoid of known overt pathogenic traits
but more likely to be resistant to even antibiotics of last resort. Effective control of multiple-drug resistant enterococci will require 1)
better understanding of the interaction between enterococci, the hospital
environment, and humans, 2) prudent antibiotic use, 3) better contact
isolation in hospitals and other patient care environments, and 4) improved
surveillance. Equally important is renewed vigor in the search for
additional drugs, accompanied by the evolution of new therapeutic paradigms
less vulnerable to the cycle of drug introduction and drug resistance.

TEXT:

The past few years have witnessed increasing interest in enterococci.
Until recently, these ordinary bowel commensals languished as misclassified
streptococci, commonly perceived "with the exception of endocarditis and
rare cases of meningitis ... as not ... a major cause of serious infection" (1). In the last decade, however, enterococci have become
recognized as leading causes of nosocomial bacteremia, surgical wound
infection, and urinary tract infection (2,3). Two types of enterococci
cause infections: 1) those originating from patients' native flora, which
are unlikely to possess resistance beyond that intrinsic to the genus and
are unlikely to be spread from bed to bed, and 2) isolates that possess
multiple antibiotic resistance traits and are capable of nosocomial
transmission. The therapeutic challenge of multiple-drug resistant (MDR)
enterococci--those strains with significant resistance to two or more
antibiotics, often including, but not limited to, vancomycin--has brought
their role as important nosocomial pathogens into sharper focus.

The accretion and spread of antibiotic resistance determinants among
enterococci, to the point where some clinical isolates are resistant to all
standard therapies, highlight both the vulnerability of our present
armament as well as the looming prospect of a "postantibiotic era" (4).
This review focuses on the magnitude and nature of the problem posed by
enterococci in general, and MDR enterococci in particular.

For many points,
only representative citations are provided.

HABITAT AND MICROBIOLOGY

Enterococci normally inhabit the bowel. They are found in the intestine of
nearly all animals, from cockroaches to humans. Enterococci are readily
recovered outdoors from vegetation and surface water, probably because of
contamination by animal excrement or untreated sewage (5). In humans,
typical concentrations of enterococci in stool are up to 108 CFU per gram
(6). Although the oral cavity and vaginal tract can become colonized,
enterococci are recovered from these sites in fewer than 20[percent] of
cases. The predominant species inhabiting the intestine varies. In Europe,
the United States, and the Far East, Enterococcus faecalis predominates in
some instances and E. faecium in others (6). Ecologic or microbial factors
promoting intestinal colonization are obscure. Of 14 or more enterococcal
species (7), only E. faecalis and E. faecium commonly colonize and infect
humans in detectable numbers. E. faecalis is isolated from approximately
80[percent] of human infections, and E. faecium from most of the rest.
Infections to other enterococcal species are rare.

Enterococci are exceedingly hardy. They tolerate a wide variety of
growth conditions, including temperatures of 10[degree]C to 45[degree]C,
and hypotonic, hypertonic, acidic, or alkaline environments. Sodium azide
and concentrated bile salts, which inhibit or kill most microorganisms, are
tolerated by enterococci and used as selective agents in agar-based media.
As facultative organisms, enterococci grow under reduced or oxygenated
conditions. Enterococci are usually considered strict fermenters because
they lack a Kreb's cycle and respiratory chain (8). E. faecalis is an
exception since exogenous hemin can be used to produce d, b, and o type
cytochromes (9,10). In a survey of 134 enterococci and related
streptococci, only E. faecalis and Lactococcus lactis expressed
cytochrome-like respiration (11). Cytochromes provide a growth advantage to
E. faecalis during aerobic growth (9). E. faecalis cytochromes are only
expressed under aerobic conditions in the presence of exogenous hemin
(9,10,12) and, therefore, may promote the colonization of inappropriate
sites.

Enterococci are intrinsically resistant to many antibiotics. Unlike
acquired resistance and virulence traits, which are usually transposon or
plasmid encoded, intrinsic resistance is based in chromosomal genes, which
typically are nontransferrable. Penicillin, ampicillin, piperacillin,
imipenem, and vancomycin are among the few antibiotics that show consistent
inhibitory, but not bactericidal, activity against E. faecalis. E. faecium
are less susceptible to b-lactam antibiotics than E. faecalis because the
penicillin-binding proteins of the former have markedly lower affinities
for the antibiotics (13). The first reports of strains highly resistant to
penicillin began to appear in the 1980s (14,15).

Enterococci often acquire antibiotic resistance through exchange of
resistance-encoding genes carried on conjugative transposons,
pheromone-responsive plasmids, and other broad-host-range plasmids (6). The
past two decades have witnessed the rapid emergence of MDR enterococci.
High-level gentamicin resistance occurred in 1979 (16) and was quickly
followed by numerous reports of nosocomial infection in the 1980s (17).
Simultaneously, sporadic outbreaks of nosocomial E. faecalis and E. faecium
infection appeared with penicillin resistance due to b-lactamase production
(18); however, such isolates remain rare. Finally, MDR enterococci that had
lost susceptibility to vancomycin were reported in Europe (19,20) and the
United States (21).

Among several phenotypes for vancomycin-resistant enterococci, VanA
(resistance to vancomycin and teicoplanin) and VanB (resistance to
vancomycin alone) are most common (22). In the United States, VanA and VanB
account for approximately 60[percent] and 40[percent] of vancomycin-resistant enterococci (VRE) isolates, respectively (23).
Inducible genes encoding these phenotypes alter cell wall synthesis and
render strains resistant to glycopeptides (22).

VanA and VanB types of resistance are primarily found among
enterococci isolated from clinical, veterinary, and food specimens (24),
but not other common intestinal or environmental bacteria. In the
laboratory, however, these genes can be transferred from

enterococci to other bacteria (22). For example, Staphylococcus aureus has been rendered vancomycin-resistant through apparent transfer of resistance from E. faecalis on the surface of membrane filters and on the skin of hairless obese mice (25), which indicates that there is no biologic barrier to the emergence of vancomycin-resistant S. aureus. Clinical isolates of highly vancomycin-resistant S. aureus have yet to be identified, although strains with reduced susceptibility to vancomycin have appeared (26). The mechanism of resistance for these strains remains undetermined but does not appear to involve genes associated with VanA or VanB phenotypes.

EPIDEMIOLOGY

Enterococci account for approximately 110,000 urinary tract infections, 25,000 cases of bacteremia, 40,000 wound infections, and 1,100 cases of endocarditis annually in the United States (2,27,28). Most infections occur in hospitals. Although several studies have suggested an increase in nosocomial infection rates for enterococci in recent years, National Nosocomial Infections Surveillance system data show little change in the percentage of enterococcal bloodstream (12[percent] vs. 7[percent]), surgical site (15[percent] vs. 11[percent]), and urinary tract (14[percent] vs. 14[percent]) infections over the past 2 decades (3,29). Adequate surveillance data prior to 1980 are not available. Enterococcal infection deaths have also been difficult to ascertain because severe comorbid illnesses are common; however, enterococcal sepsis is implicated in 7[percent] to 50[percent] of fatal cases (6). Several case-control and historical cohort studies show that death risk associated with antibiotic-resistant enterococcal bacteremia is severalfold higher than death risk associated with susceptible enterococcal bacteremia (30). This trend will likely increase as MDR isolates become more prevalent.

Colonization and infection with MDR enterococci occur worldwide. Early reports showed that in the United States, the percentage of nosocomial infections caused by VRE increased more than 20-fold (from 0.3[percent] to 7.9[percent]) between 1989 and 1993, indicating rapid dissemination. New database technologies, such as The Surveillance Network (TSN) Database-USA, now permit the assessment of resistance profiles according to species. TSN Database electronically collects and compiles data daily from more than 100 U.S. clinical laboratories, identifies potential laboratory testing errors, and detects emergence of resistance profiles and mechanisms that pose a public health threat (e.g., vancomycin-resistant staphylococci).

Data collected by the TSN Database between 1995 and September 1, 1997 were analyzed to determine whether the earlier increase in vancomycin resistance was unique to vancomycin, whether it represented a continuing trend, and whether speciation is quantifiably important in analyzing this trend. E. faecalis resistance to ampicillin and vancomycin is uncommon (Figure 1); little change in resistance prevalence occurred from 1995 to 1997. In contrast, E. faecium vancomycin and ampicillin resistance increased alarmingly. In 1997, 771 (52[percent]) of 1,482 of E. faecium isolates exhibited vancomycin resistance, and 1,220 (83[percent]) of 1,474 isolates exhibited ampicillin resistance (Figure 1). E. faecium resistance notwithstanding, E. faecalis remained by far the most commonly encountered of the two enterococcal species in TSN Database. E. faecalis to E. faecium total isolates were approximately 4:1 (Figure 1), blood isolates 3:1, and urine isolates 5:1. This observation underscores important differences in the survival strategies and likelihood of therapeutic success, critical factors usually obscured by lumping the organisms together as Enterococcus species or enterococci. Widespread emergence and dissemination of ampicillin and vancomycin resistance in E. faecalis would significantly confound the current therapeutic dilemma. There is little reason to suspect that vancomycin and ampicillin resistances only provide selective advantage for the species faecium and not faecalis. The relative absence of these resistances in E. faecalis may simply reflect a momentary lack of penetrance and equilibration of the traits. Because of these important differences between the two species, meaningful surveillance of

enterococcal resistance must include species identification.

Although exact modes of nosocomial transmission for MDR enterococci are difficult to prove, molecular microbiologic and epidemiologic evidence strongly suggest spread between patients, probably on the hands of health-care providers or medical devices, and between hospitals by patients with prolonged intestinal colonization. At least 16 outbreaks of MDR enterococci have been reported since 1989 (31); all but two were due to E. faecium. This disparity, particularly in view of the higher numbers of clinical E. faecalis isolates, may reflect a reporting bias due to the novelty of the combinations of resistance that occur in E. faecium. When isolates from outbreaks of MDR enterococci have been analyzed by genetic fingerprints, more than half involve clonally related isolates (18,32).

Prior treatment with antibiotics is common in nearly all patients colonized or infected with MDR enterococci (33-35). Clindamycin, cephalosporin, aztreonam, ciprofloxacin, aminoglycoside, and metronidazole use is equally or more often associated with colonization or infection with MDR enterococci than vancomycin use. Other risk factors include prolonged hospitalization; high severity of illness score; intraabdominal surgery; renal insufficiency; enteral tube feedings; and exposure to specific hospital units, nurses, or contaminated objects and surfaces within patient-care areas.

INFECTION CONTROL

Controlling the spread of MDR enterococci among inpatients is difficult. We know relatively little about the biology of enterococcal transmission or the specific microbial factors favoring colonization by exogenous enterococcal strains. Nevertheless, VRE infection control policies, which could apply to MDR enterococci, were recently published by the Hospital Infection Control Practices Advisory Committee (36). Control methods include routine screening for vancomycin resistance among clinical isolates, active surveillance for VRE in intensive care units, contact isolation to minimize person-to-person transmission, and vancomycin restriction.

These measures to limit VRE spread, however, have failed on occasion (35). Not all hospitals can or are willing to perform active surveillance. Because more patients are typically colonized with VRE (3[percent] to 47[percent]) than are infected (35,37,38), and because intestinal colonization can be prolonged, passive surveillance by routine cultures allows colonized inpatients to go unidentified and serve as point sources for continued spread of VRE. Even if all colonized inpatients are successfully identified, VRE may be spread by health-care workers through either inadequate hand washing (39) or through contact with items such as bedrails, sinks, faucets, and doorknobs (enterococci can persist for weeks on environmental surfaces) (40). Decontamination efforts must be rigorous.

The Hospital Infection Control Practices Advisory Committee strongly recommended restricting oral and parenteral vancomycin to control VRE (36). However, limiting use of vancomycin while ignoring widespread use of other broad spectrum antibiotics likely will not lead to maximal control of VRE or of MDR enterococci.

Antibiotics may promote colonization and infection with MDR enterococci by at least two mechanisms. First, many broad spectrum antibiotics have little or no anti-enterococcal activity, and administration commonly leads to overgrowth of susceptible (or resistant) enterococci at sites at risk for infection. Second, most antibiotics substantially reduce the normal resistance of the intestinal tract to colonization by exogenous organisms (41). Colonization resistance results primarily from the "limiting action" of the normal anaerobic flora, and to a lesser extent from an intact mucosa, gastric acid secretion, intestinal motility, and intestinal-associated immunity (41). Antibiotic-induced alterations in the protective flora of the intestine provide large footholds for colonization with exogenous pathogens such as MDR enterococci (41). Antibiotic restriction programs would be more effective if they included prudent prescribing of all antibiotics, not just single agents such as vancomycin. This approach substantially decreased intestinal colonization with VRE in one hospital pharmacy that restricted

vancomycin, cefotaxime, and clindamycin (42).

At a minimum, a successful program for control of MDR enterococci requires effective passive and active surveillance to identify colonized and infected patients, absolute adherence to contact isolation by health-care workers, rigorous decontamination of patient-contact areas and judicious use or restriction of vancomycin and other broad spectrum antibiotics.

THERAPEUTIC APPROACHES

Suitable antibiotics often are not available to treat MDR enterococcal infections, e.g., endocarditis or bacteremia, in the presence of neutropenia. Combinations of penicillin with vancomycin, ciprofloxacin with ampicillin, or novobiocin with doxycycline, among others, have been used (43) but can be unpredictable and remain clinically unproven. In one report chloramphenicol successfully treated chloramphenicol-susceptible infections (44), but these findings await confirmation in controlled trials.

Promising new antibiotics for MDR enterococcal infection under investigation include fluoroquinolones, streptogramins, oxazolidinones, semisynthetic glycopeptides, and glycylcyclines. Clinafloxacin, a fluoroquinolone with improved potency against enterococci compared with ciprofloxacin, has excellent activity against VRE, appears bactericidal in vitro, and has been effective in treatment of enterococcal infections in a murine model (45). Although single-step resistance to clinafloxacin could not be detected in vitro, multistep resistance is readily achieved. Should this agent gain approval for treatment of enterococcal infections, selection for resistance may limit its effectiveness.

Quinupristin/dalfopristin (Synercid) is a combination of streptogramins A and B that inhibits protein synthesis and has a narrower spectrum of activity against enterococci than clinafloxacin (46). Many, but not all, E. faecium isolates with VanA and VanB phenotypes are susceptible (47); however, E. faecalis is uniformly resistant, and superinfection has been reported during therapy (48). In addition, quinupristin/dalfopristin is bacteriostatic only, potentially allowing emergence of resistance (49). For these reasons the drug may have only a limited role in treating MDR enterococcal infections. Novel oxazolidinones and glycylcyclines have also shown potent activity against enterococci, including MDR enterococci (50,51), but await further testing.

The substantial drawback of the broad spectrum approach is that the more organisms affected (both protective commensals as well as pathogens), the more opportunities for resistance to evolve. Broad spectrum antibiotics permit empiric therapy in the absence of a specific diagnosis and generate a more substantial return on investment in the short term. However, broad spectrum antibiotics affect not only disease-causing organisms but also commensals present in numbers large enough to generate resistance by otherwise rare mutations or genetic exchange events. As long as market forces favor development of broad spectrum therapeutics, a cycle of drug introduction followed by emergence of resistance undoubtedly will continue.

TARGETED THERAPEUTICS

In contrast to the historical reliance on broad spectrum antibiotic therapy, the continuing development and introduction of rapid diagnostic techniques (52) may allow a more focused approach to infectious disease therapy. Any of the myriad microbial-host interactions that subvert the host response or damage tissues during an infection represent potential therapeutic targets. However, many key interactions in disease pathogenesis are specific to the organism involved--a characteristic that is both a strength and a weakness. Because of the specificity of these interactions, rapid and accurate diagnosis is required. However, therapeutics aimed only at interaction between host and a specific pathogen should leave the diverse commensal flora essentially unaffected. As a result, the targeted population would be restricted to the relatively small numbers of disease-producing bacteria and would not likely reach the numbers or diversity required to make development of resistance a statistical probability.

The current spectrum of approaches to identify new antiinfective compounds has two extremes: 1) screening vast libraries of compounds to

identify substances that by chance inhibit a microbial property and 2) detailed study of interactions between host and parasite to identify critical events leading to host tissue damage or compromise (53).

With a long-term view toward new therapeutic approaches as well as optimal use of existing therapies, we and others have begun examining in detail the interactions between enterococci and host (6). A major obstacle is that enterococci also form part of the commensal or autochthonous flora; as such, they are nearly devoid of traits traditionally associated with overt pathogens and have subtle interactions with the host. Using inocula with as few as 10 organisms, we have developed sensitive biologic systems for examining the host-parasite interactions (54).

Although E. faecium strains are resistant to vancomycin and ampicillin more often than E. faecalis strains, the relative proportion of infections caused by these species has not dramatically changed in recent years (Figure 1). Since both organisms are frequently isolated from the commensal flora, this bias suggests that E. faecalis traits confer a greater degree of intrinsic virulence, for example, cytolysin production, pheromone-responsive plasmid transfer (and accompanying production of aggregation substance), extracellular superoxide production, and a newly identified surface protein tentatively termed Esp (5,56,57) (Figure 2). These properties provide logical points of departure for developing new targeted therapeutic approaches to enterococcal disease; examination of more subtle interactions between E. faecium and host will follow as an understanding of enterococcal biology evolves.

TARGETING THE E. FAECALIS CYTOLYSIN

Cytolysin is disproportionately expressed by E. faecalis strains associated with disease (5,55,56). This cytolysin causes rupture of a variety of target membranes, including bacterial cells, erythrocytes, and other mammalian cells, with activity observed as a hemolytic zone on some types of blood agar. Cytolysin contributes to the toxicity or lethality of infection in several infection models and is associated with a fivefold increased risk of sudden death from nosocomial bacteremia (54,56-59). Cytolysin also contributes to the appearance of enterococci in a murine bacteremia model (Figure 3; 45,60), an observation consistent with the disproportionate representation of cytolytic strains among human blood isolates (56,62).

Beginning with E.W. Todd in 1934 (63) and culminating in a recent study (64), the E. faecalis cytolysin has now been characterized as a unique, extensively modified bacterial toxin (Figure 4). The cytolysin maturation pathway is ideally designed for therapeutic targeting because the two structural subunits are activated by an extracellular protease, an event that is accessible and potentially inhibitable by a novel therapeutic. Moreover, the activator protease, CylA, belongs to the subtilisin class of serine proteases (64), whose structure-function relationships and inhibitor design we are beginning to understand.

Investigations are in progress to design and test inhibitors of extracellular cytolysin activation to determine whether a reduction by several logs in the levels of circulating enterococci can be attained as would be predicted by the observed behavior of cytolysin mutants (Figure 3).

An inhibitor of cytolysin activation, accompanied by appropriate rapid diagnostics, would be of potential value in treating bacteremia caused by cytolytic strains of E. faecalis without affecting commensal flora. Development of resistance should be exceedingly improbable because of the small number of bacteria targeted and because unlike antibiotics, cytolysin inhibitors would not act directly on the organism itself.

OTHER ENTEROCOCCAL TARGETS

Several laboratories are using information on the E. faecalis genome and genomes of other pathogens to identify therapeutic targets (66) and facilitate studies on pathogenesis for the remaining 60[percent] of noncytolytic enterococcal infections. The genome of an E. faecalis strain that caused multiple hospital infections (56) was sampled at high frequency by sequence analysis. Several sequences appeared to have a role in host-parasite interaction. The gene specifying Esp encodes an apparent surface protein of unusual repeating structure (67). Although

a role for this protein in enterococcal infection has yet to be determined, its distribution among clinical and commensal strains is tantalizing: 29 of 30 strains with this gene were recovered from patients with bacteremia or endocarditis; one of 34 isolates obtained from healthy volunteers possessed Esp. The core of this large protein (inferred mass of 202 kDa) consists of a series of 82 amino acid repeats encoded by highly conserved tandem 246 base pair repeats. Lack of divergence in repeat sequences suggests that recombination occurs at high frequency, perhaps during infection. Moreover, the number of repeats observed in homologous genes from different E. faecalis isolates is 3 to 9 (67). This gene is flanked by a sequence similar to the transposase of IS905. None of 24 clinical or laboratory E. faecium isolates had this gene (67; V. Shankar, G. Lindahl, and M. Gilmore, unpub. data).

A second promising lead involves a series of genes encoding products highly related to enzymes involved in O-antigen synthesis in gram-negative bacteria (68). Preliminary evidence suggests that in E. faecalis these genes contribute to cell wall carbohydrate synthesis and that this carbohydrate relates to persistence in vivo. A knockout in one of these genes results in a strain with normal in vitro growth, but after subcutaneous injection, the mutant was more readily cleared than the wild type parental strain (68). One of the genes studied was present in all E. faecalis strains examined, whereas another occurs only in E. faecalis strains that share a periodate-susceptible epitope (68). Collectively, these data indicate that enzymes for synthesis of E. faecalis surface carbohydrates are important for persistence in vivo and may represent a useful therapeutic target. Taking a different approach, Arduino et al. (69,70) identified a protease-resistant, periodate susceptible substance associated with some strains of E. faecium, but not E. faecalis, which conferred resistane to phagocytosis in vitro. The relationship between the putative carbohydrate of E. faecalis under study above and the inhibitory substance of E. faecium remains to be determined. It may be found that many enterococci produce such carbohydrates at biologically significant levels in vivo, but only some strains of E. faecium do so in vitro.

Finally, recent observations indicate that nearly all E. faecalis strains, and only a few E. faecium strains, generate substantial extracellular superoxide. When E. faecalis isolates from patients with endocarditis and bacteremia were compared with isolates from healthy volunteers (71), on average, extracellular superoxide production was 60[percent] higher among blood isolates than commensal strains. These data raised several questions: Do E. faecalis that produce larger amounts of extracellular superoxide possess greater metabolic flexibility, facilitating adaptation to nonintestinal infection sites? Does free radical production lead to host cell damage, allowing release of normally sequestered nutrients (e.g., hemin) that might promote enhanced E. faecalis growth through cytochrome formation? Might antioxidants modulate colonization or invasive infection? Answers to these questions may provide new insights into the transition from intestinal colonization to infection and may suggest new preventive strategies.

OBSTACLES TO FURTHER DEVELOPMENT

Although important insights into enterococcal biology and pathogenesis are being gleaned from a reverse genetic approach, a paucity of information still exists on how enterococci colonize the intestinal tract and cause infection. For example, do E. faecalis or E. faecium colonize the colon through specific interactions with ligands on human epithelial cells or intestinal mucin? Do MDR enterococci possess alternate binding activities that enable them to colonize the intestinal tract at new sites without competing with the indigenous enterococci? Do probiotics have a role in restoring colonization resistance to an intestinal ecology altered by broad spectrum antibiotics?

Is enough being done to combat the emergence of highly resistant nosocomial pathogens? To effectively compete, industry remains highly responsive to market opportunities. Research in the public sector has been slow to respond, and as a result, our understanding of the biology of

enterococcal infection is inadequate. Reasons for the modest public sector
response include the following. 1) The emergence of resistant enterococci
coincided with a reduction of public support for non-AIDS related
infectious disease research. 2) The pathogenesis of nosocomial infection
deviates from paradigms established for obligate pathogens. 3) The research
infrastructure is relatively small because of the low importance
traditionally attached to enterococci as etiologic agents of human disease
and the deemphasis on antibiotic resistance research in the 1980s.

CONCLUSIONS

Historically, substantial resources have been invested in developing an
in-depth understanding of the molecular biology of model organisms. During
the 1960s and 1970s, when gram-negative organisms were leading causes of
hospital- and community-acquired infections and gram-positive organisms
were typically sensitive to existing antibiotics (72), a sizable investment
in gram-negative model organisms was appropriate. However, with the
emergence of gram-positive organisms as leading causes of both hospital-
and community-acquired infection in the 1990s, a reevaluation of public
research priorities is warranted.

Since antibiotic use became widespread 50 years ago, bacteria have
steadily and routinely developed resistance. Control of the emergence of
resistance will depend on new approaches to prudent antibiotic use in
hospitals and clinics, based in part on improved surveillance for MDR
enterococci and on better systems to encourage staff adherence to contact
isolation procedures. Equally important will be development of new drugs
with narrower spectra of activity aimed at known and potentially new
targets and the evolution of market conditions that favor their use.

Added material

Mark M. Huycke

Dr. Huycke is an associate professor in the Infectious Diseases
Section, Department of Medicine, Oklahoma University Health Sciences
Center. He is interested in enterococcal pathogenesis as it relates to
extracellular superoxide production by E. faecalis.

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Figure 1. Epidemiology of enterococcal infection based on 15,203
susceptibility results obtained by The Surveillance Network (TSN)
Database-USA, 1995 to Sep 1, 1997. The increase in total numbers between
1995 and 1996 represents additional reporting centers coming on line.
Numbers for 1997 represent total collected for the partial year to Sep 1,
1997.

Figure 2. Virulence traits and their association with enterococcal
species.

Figure 3. Cytolysin favors the appearance of circulating enterococci.
In this experiment, 107 CFU of E. faecalis, either cytolytic FA2-2(pAM714)
(60) or noncytolytic FA2-2(pAM771) (64), were intraperitoneally injected
(45) into groups of five BalbC mice. Viable bacteria in liver, spleen, and
the bloodstream were enumerated 48 hrs following injection, and
significance assessed by Student's t-test. (P. Coburn, L.E. Hancock, and
M.S. Gilmore, in preparation).

Figure 4. Cytolysin is expressed and processed through a complex
maturation pathway (64). The cytolysin precursors, CylLL and CylLS, are
ribosomally synthesized. The putative modification protein, CylM, is
required for the expression of CylLL and CylLS in an activatable form, and

the secreted forms, CylLL and CylLS were recently shown to possess the amino acid lanthionine as the result of posttranslational modification (64). CylLL and CylLS both are secreted by CylB (65), which is accompanied by an initial proteolytic trimming event (64) converting each to CylLL' and CylLS', respectively. Once secreted, CylLL' and CylLS' are both functionally inactive until six amino acids are removed from each amino terminus. This final step in maturation is catalyzed by CylA (64), a subtilisin-type serine protease. Since this final catalytic event is essential, occurs extracellularly, and is catalyzed by a class of enzyme for which a substantial body of structural information exists, it represents an ideal therapeutic target. As shown in Figure 3, inhibition of cytolysin by mutation (or potentially by therapeutic intervention) results in a reduction by several orders of magnitude in the number of circulating organisms.

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Dis 1996;173:743-6
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increasingly limited therapies. Proc Natl Acad Sci U S A 1994;91:2420-7.

DESCRIPTORS:
Bacteria--Multidrug resistance; Enterococcus

6/9/50 (Item 50 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

03321621 BIOSIS NO.: 000072049725
BACTERIAL SURVIVAL IN A DILUTE ENVIRONMENT
AUTHOR: SJOGREN R E; GIBSON M J
AUTHOR ADDRESS: DEP. MICROBIOL. AND BIOCHEM., UNIV. VERMONT, BURLINGTON, VERMONT 05405.
JOURNAL: APPL ENVIRON MICROBIOL 41 (6). 1981. 1331-1336. 1981
FULL JOURNAL NAME: Applied and Environmental Microbiology
CODEN: AEMID
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: Bacteria were isolated from lake water and their ability to
remain viable in a dilute, nutrient-deficient environment was tested by a
method that permits suspension of test bacteria between 2 appressed
microporous membranes in an aqueous environment. This approach permitted
separation of the lake isolates into 2 categories. Members of the tribe
Klebsielleae had a prolonged survival rate of 40% or better after 24 h;
nonsurvivors were not viable for much longer than 24 h. These
nonsurvivors belonged to the genera Acinetobacter, Aeromonas,
Alcaligenes, Erwinia, Escherichia, Flavobacterium and Pseudomonas.
Differences in RNase and ATPase levels between Escherichia coli
(nonsurvivor) and Klebsiella (survivor) cells were detected. At pH 7.5,
stressed E. coli cells contained 14% of the ATPase activity detected in
the control; at pH 5.5, in the presence of Ca ions, these same cells
contained 50% of the control ATPase levels. At pH 7.2, E. coli cells were
strongly inhibited by an ATPase inhibitor, bathophenanthroline (88%);
oligomycin (64%); and the proton ionophore carbonyl
cyanide-m-chlorophenyl hydrazone (67%). Sodium azide and valinomycin
were only moderately inhibitory (15 and 28%, respectively). Although the
ability to scavenge internal endogenous reserves seems important, certain
enteric bacteria seem capable of using acidic conditions (pH 5.5) as an
electrochemical gradient to generate necessary high-energy intermediates
for prolongation of survival beyond that possible in environments of
near-neutral pH.

DESCRIPTORS: KLEBSIELLEAE ACINETOBACTER

AEROMONAS ALCALIGENES ERWINIA
ESCHERICHIA FLAVOBACTERIUM PSEUDOMONAS
ESCHERICHIA-COLI LAKE WATER PH
ATPASE ACTIVITY
CONCEPT CODES:
07514 Ecology; Environmental Biology-Limnology
30000 Bacteriology, General and Systematic
31000 Physiology and Biochemistry of Bacteria
37015 Public Health: Environmental Health-Air, Water and Soil Pollution
07517 Ecology; Environmental Biology-Water Research and Fishery Biology
(1969-1984)
10010 Comparative Biochemistry, General
10050 Biochemical Methods-General
10060 Biochemical Studies-General
10064 Biochemical Studies-Proteins, Peptides and Amino Acids
10502 Biophysics-General Biophysical Studies
10506 Biophysics-Molecular Properties and Macromolecules
10508 Biophysics-Membrane Phenomena
10802 Enzymes-General and Comparative Studies; Coenzymes
10804 Enzymes-Methods
10806 Enzymes-Chemical and Physical
10808 Enzymes-Physiological Studies
13002 Metabolism-General Metabolism; Metabolic Pathways
13003 Metabolism-Energy and Respiratory Metabolism
13012 Metabolism-Proteins, Peptides and Amino Acids
13014 Metabolism-Nucleic Acids, Purines and Pyrimidines
13202 Nutrition-General Studies, Nutritional Status and Methods
13203 Nutrition-Malnutrition; Obesity
22003 Pharmacology-Drug Metabolism; Metabolic Stimulators
32000 Microbiological Apparatus, Methods and Media
37400 Public Health: Microbiology
38502 Chemotherapy-General; Methods; Metabolism
BIOSYSTEMATIC CODES:
04000 Bacteria-Unspecified (1979-)
04716 Pseudomonadaceae (1979-)
04720 Gram-negative Aerobic Rods and Cocci-Uncertain Affiliation (1979-)
04810 Enterobacteriaceae (1979-)
04812 Vibrionaceae (1979-)
04814 Gram-negative Facultatively Anaerobic Rods-Uncertain Affiliation (1979-)
05110 Neisseriaceae (1979-)
BIOSYSTEMATIC CLASSIFICATION (SUPER TAXA):
Microorganisms
Bacteria

6/9/48 (Item 48 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

04353539 BIOSIS NO.: 000078083083
EFFECTS OF METABOLIC INHIBITORS ON THE ALCOHOLIC FERMENTATION BY SEVERAL YEASTS IN BATCH OR IN IMMOBILIZED CELL SYSTEMS
AUTHOR: AMIN G; STANDAERT P; VERACHTERT H
AUTHOR ADDRESS: LAB. INDUSTRIAL MICROBIOL. BIOCHEM., UNIV. LEUVEN, KARDINAAL MERCIERLAAN, 92 B-3030 HEVERLEE-LOUVAIN, BELGIUM.
JOURNAL: APPL MICROBIOL BIOTECHNOL 19 (2). 1984. 91-99. 1984
CODEN: EJABD
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: In previous papers it was shown that the bacterium Zymomonas mobilis might be an interesting alternative for industrial alcohol production from sugar, compared to Saccharomyces bayanus. Factors that might increase the glucose to ethanol conversion efficiency and which are in favor of the bacterium, are the production of less biomass and less by-products such as glycerol, succinic acid, butanediol, acetoin and acetic acid. In order to reduce the synthesis of biomass, 3 metabolic inhibitors were now studied: dinitrophenol, azide and arsenate. Their effects on the alcoholic fermentation in batch and in immobilized cell system were investigated, using 3 yeasts: S. bayanus, Schizosaccharomyces pombe and S. diastaticus. Dinitrophenol in 0.1 mM concentration was effective in increasing the conversion of glucose to ethanol especially with S. bayanus while azide in 0.1 mM concentration was better with S. pombe. In immobolized systems high steady state ethanol production from 15% glucose media was obtained by inclusion into the media of dinitrophenol or azide . Arsenate had less effect at the concentrations used. As a result, ethanol productivity in grams per hour was increased from around 70 in the absence of inhibitor to around 74 in the presence of dinitrophenol with S. bayanus. With S. pombe the productivity was increased from around 65 in the absence of inhibitor to around 74 in the presence of azide . The specific ethanol productivity expressed as 1 g ethanol formed per hour and per gram viable cells was increased from 0.87 to 1.37 for S. pombe and from 1.02 to 1.66 for S. bayanus.

DESCRIPTORS: ZYMOMONAS-MOBILIS
SACCHAROMYCES-BAYANUS
SACCHAROMYCES-DIASTATICUS
SCHIZOSACCHAROMYCES-POMBE BIOMASS
ETHANOL
PRODUCTIVITY
CONCEPT CODES:
10511 Biophysics-Bioengineering
13002 Metabolism-General Metabolism; Metabolic Pathways
39007 Food and Industrial Microbiology-Biosynthesis, Bioassay and
Fermentation
51510 Plant Physiology, Biochemistry and Biophysics-Growth,
Differentiation
51519 Plant Physiology, Biochemistry and Biophysics-Metabolism
02504 Cytology and Cytochemistry-Plant
10010 Comparative Biochemistry, General
10050 Biochemical Methods-General
10060 Biochemical Studies-General
10068 Biochemical Studies-Carbohydrates
13003 Metabolism-Energy and Respiratory Metabolism
13004 Metabolism-Carbohydrates
31000 Physiology and Biochemistry of Bacteria
32000 Microbiological Apparatus, Methods and Media
51524 Plant Physiology, Biochemistry and Biophysics-Apparatus and
Methods
BIOSYSTEMATIC CODES:
04814 Gram-negative Facultatively Anaerobic Rods-Uncertain
Affiliation (1979-)
15100 Ascomycetes
BIOSYSTEMATIC CLASSIFICATION (SUPER TAXA):
Microorganisms
Bacteria
Plants
Nonvascular Plants
Fungi

6/9/47 (Item 47 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

05135169 BIOSIS NO.: 000081093294
INFLUENCE OF ENDOGENOUS CATALASE ACTIVITY ON THE SENSITIVITY OF THE ORAL
BACTERIUM
ACTINOBACILLUS-ACTINOMYCETEMCOMITANS AND THE ORAL HAEMOPHILI TO
THE BACTERICIDAL PROPERTIES OF HYDROGEN PEROXIDE
AUTHOR: MIYASAKI K T; WILSON M E; ZAMBON J J; GENCO R J
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JOURNAL: ARCH ORAL BIOL 30 (11-12). 1985 (RECD. 1986). 843-848. 1985
FULL JOURNAL NAME: Archives of Oral Biology
CODEN: AOBIA
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: Actinobacillus actinomycetemcomitans and the genetically-related
oral haemophili (Haemophilus segnis, Haemophilus aprhophilus and
Haemophilus paraphrophilus) exhibit a range of sensitivities to the
lethal effect of hydrogen peroxide (H2O2), A. actinomycetemcomitans being
the most resistant. To extend this information, susceptibility to a range
of H2O2 concentrations (10-6-10-3 M) was assessed by incubating bacterial
suspensions for 1 h at 37.degree. C in the presence of H2O2 and spreading
the suspensions on chocolate agar plates to determine the concentration
of H2O2 producing a 50 per cent reduction in colony-forming units (LD50).
Catalase activity was quantified with a Clark-type oxygen electrode,
which polarographically monitored the formation of dissolved oxygen in
bacterial suspensions on sonicates following addition of reagent H2O2.
Sensitivity to H2O2 did not correlate with catalase activity, either in
intact cells or in bacterial sonicates. Specifically, some bacterial
strains with undetectable catalase activity were highly resistant to
H2O2. Micromolar concentrations of sodium azide which completely
inhibited cell-associated catalase activity did not affect the resistance
of A. actinomycetemcomitans to H2O2. Thus, the endogenous catalase
activity of A. actinomycetemcomitans and certain oral haemophili is not
an important determinant of resistance to the bactericidal effects of
H2O2.

DESCRIPTORS: HAEMOPHILUS-SEGNIS
HAEMOPHILUS-APHROPHILUS
HAEMOPHILUS-PARAPHROPHILUS
CONCEPT CODES:
10808 Enzymes-Physiological Studies
13002 Metabolism-General Metabolism; Metabolic Pathways
13012 Metabolism-Proteins, Peptides and Amino Acids
19006 Dental and Oral Biology-Pathology
36002 Medical and Clinical Microbiology-Bacteriology
10060 Biochemical Studies-General
10064 Biochemical Studies-Proteins, Peptides and Amino Acids

19001 Dental and Oral Biology-General; Methods
BIOSYSTEMATIC CODES:
04814 Gram-negative Facultatively Anaerobic Rods-Uncertain
Affiliation (1979-)
BIOSYSTEMATIC CLASSIFICATION (SUPER TAXA):
Microorganisms
Bacteria

6/9/45 (Item 45 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

05581313 BIOSIS NO.: 000083054453
STUDIES ON IMMUNOGENICITY OF PASTEURELLA-MULTOCIDA ISOLATED FROM SWINE IN
KOREA
AUTHOR: KIM J Y; PARK J M; KIM O N
AUTHOR ADDRESS: VET. RES. INST., ANYANG, KOREA.
JOURNAL: RES REP RURAL DEV ADM (SUWEON) 28 (LIVEST. AND VET.). 1986. 77-93.
1986
FULL JOURNAL NAME: Research Reports of the Rural Development Administration
(Suweon)
CODEN: NSYNE
RECORD TYPE: Abstract
LANGUAGE: KOREAN

ABSTRACT: Pasteurella multocida plays an important role in inducing
respiratory disease of pigs. This acts not only as a primary invading
organism during the early stages of rearing period but also as a
secondary invader to primary organisms such as Bordetella bronchiseptica,
Haemophilus spp., or Mycoplasma spp. In this study, isolation and
identification of P. multocida were attempted from lung samples and nasal
swabs from pigs. Serotyping was performed against capsular and somatic
antigen on the isolates and also immunogenicity of P. multocida isolated
from pneumonic pigs was tested to develop a vaccine against P. multocida.
The results obtained are as follows. A total of 127 (23.7%) P. multocida
were isolated from 536 specimens collected from slaughtered pigs and
piglets showing respiratory signs. Of 127 P. multocida, 95 isolates were
from 414 cases of pneumonic lungs of slaughtered pigs and 32 from nasal
swab specimens of 122 piglets infected with respiratory disease. Capsular
serotyping performed on the 127 P. multocida revealed that 47 strains
(37.0%) were A type (Carter's) and 38 strains (29.9%) were D type and the
remainder were untypable. When serotyping was performed against somatic
antigen on the 85 strains capsular types of which were identified as
described above 14, 15, 5, 11 and 19 strains belonged to 1A, 3A, 5A, 2D
and 4D, respectively. Among antigens prepared by various inactivation
methods; heat, formalin, phenol, sodium azide or merthiolate, formalin
treated antigen was found to the most immunogenic in mice, i.e. 94 per
cent of mice inoculated with the antigen were protected against P.
multocida challenged. In the cross immunity test between P. multocida
serotype A or D, 79 to 100 per cent were protected against homologous
challenge, while 50 to 73 per cent were protected against heterologous
challenge. The mouse protection rates of formalin treated antigen
containing incomplete freund's adjuvant (IFA), aluminum hydroxide-gel
(AHG) or both IFA and AHG were 92, 83 and 84 per cent, respectively.
Divalent antigen containing of serotype A and D protected 97 and 94 per
cent of mice when used with adjuvants respectively with AHG and IFA,
while the antigen gave 64 per cent without adjuvant. The antigen which
induced 93 to 94 per cent protection in mice gave 82 to 87 per cent and
44 to 50 per cent protection when inoculated with 0.56 .times. 109 cells
and 0.11 .times. 109 cells of its original, respectively. All the pigs
immunized with the experimental antigen were protected from challenge
exposure, while 50 to 75 per cent of pigs survived when one fifth of the
recommended does was used. When the pigs were inoculated with divalent
antigen twice at 55 and 70 days of age, the antibody titers at 3 week
post-inoculation were 20 by passive mouse protection (PMP) test and 133
to 160 by indirect haemagglutination (IHA) test. Sows were vaccinated
twice with the adjuvanted antigen at 30 to 15 days before paturition and
then sera were collected to antibody determination. The antibody titers
at paturition were 40 by PMP test and 160 to 320 by IHA test. It was
found that antibody titers of colostrum were higher than those of from

dams. Passive antibody titers to P. multicoida in piglets were 30 by PMP
and 60 by IHA test at 10 days of age and gradually disappeared as age
increased the dropped under detectable level by 30 days of age.

DESCRIPTORS: BORDETELLA-BRONCHISEPTICA HAEMOPHILUS-SPP MYCOPLASMA-SPP IMMUNIZATION CHALLENGE PROTECTION ANTIBODY RESPONSE
CONCEPT CODES:
13012 Metabolism-Proteins, Peptides and Amino Acids
16006 Respiratory System-Pathology
22018 Pharmacology-Immunological Processes and Allergy
34504 Immunology and Immunochemistry-Bacterial, Viral and Fungal
36002 Medical and Clinical Microbiology-Bacteriology
38006 Veterinary Science-Microbiology
13004 Metabolism-Carbohydrates
16001 Respiratory System-General; Methods
30500 Morphology and Cytology of Bacteria
31000 Physiology and Biochemistry of Bacteria
34502 Immunology and Immunochemistry-General; Methods
38004 Veterinary Science-Pathology
BIOSYSTEMATIC CODES:
04720 Gram-negative Aerobic Rods and Cocci-Uncertain Affiliation (1979-)
04814 Gram-negative Facultatively Anaerobic Rods-Uncertain Affiliation (1979-)
09112 Mycoplasmataceae (1979-)
85740 Suidae
BIOSYSTEMATIC CLASSIFICATION (SUPER TAXA):
Microorganisms
Bacteria
Animals
Chordates
Vertebrates
Nonhuman Vertebrates
Mammals
Nonhuman Mammals
Artiodactyls

6/9/42 (Item 42 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

10833774 BIOSIS NO.: 199799454919
Expression of the Zymomonas mobilis gfo gene for NADP-containing glucose:
Fructose oxidoreductase (GFOR) in Escherichia coli: Formation of
enzymatically active preGFOR but lack of processing into a stable
periplasmic protein.
AUTHOR: Wiegert Thomas; Sahm Hermann; Sprenger Georg A(a)
AUTHOR ADDRESS: (a)Inst. Biotechnol. 1, Forschungszentrum Juelich GmbH,
Postfach 1913, D-52425 Juelich**Germany
JOURNAL: European Journal of Biochemistry 244 (1):p107-112 1997
ISSN: 0014-2956
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Glucose:fructose oxidoreductase (GFOR) of the gram-negative
bacterium Zymomonas mobilis is a periplasmic enzyme with tightly bound
cofactor NADR The preprotein carries an unusually long Nterminal signal
peptide of 52 amino acid residues. Expression of the gfo gene in cells of
Escherichia coli K12, under the control of a tac promoter, led to
immunologically detectable proteins in western blots. and to the
formation of an enzymatically active precursor form (preGFOR), located in
the cytosol. Processing of preGFOR to the mature form was not observed in
E. coli. Replacement of the authentic GFOR signal peptide by the shorter
signal peptides of PhoA or OmpA from E. coli led to processing of the
respective GFOR precursor proteins. However. the processed proteins were
unstable and rapidly degraded in the periplasm unless an E. coli mutant
was used that carried a triple lesion for periplasmic and outer-membrane
proteases. When fusion-protein export was inhibited by sodium azide or
carboxylcyanide mchlorophenylhydrazone, the cytoplasmic precursor forms
of the respective preGFOR were not degraded. A major protease-resistant
GFOR peptide from the OmpA-GFOR fusion was found within spheroplasts of
E. coli to which NADP had been added externally. The formation of this
peptide did not occur in the presence of NAD. It is concluded that NADP
is required for GFOR to fold into its native conformation and that its
absence from the E. coli periplasm is responsible for failure to form a
stable periplasmic protein. The results strongly suggest that, in Z.
mobilis, additional protein factors are required for the transport of
NADP across the plasma membrane and/or incorporation of NADP into the
GFOR apoenzyme.

REGISTRY NUMBERS: 53-59-8: NADP; 50-99-7:

GLUCOSE; 94949-35-6:
GLUCOSE:FRUCTOSE OXIDOREDUCTASE
DESCRIPTORS:
MAJOR CONCEPTS: Biochemistry and Molecular Biophysics; Enzymology
(Biochemistry and Molecular Biophysics); Genetics; Physiology
BIOSYSTEMATIC NAMES:
Enterobacteriaceae--Eubacteria, Bacteria;
Facultatively Anaerobic Gram-Negative Rods--Eubacteria, Bacteria
ORGANISMS: facultatively anaerobic gram-negative rods (Facultatively
Anaerobic Gram-Negative Rods); Escherichia coli (Enterobacteriaceae)
; Zymomonas mobilis (Facultatively Anaerobic Gram-Negative Rods)
BIOSYSTEMATIC CLASSIFICATION (SUPER TAXA): bacteria; eubacteria;
microorganisms
CHEMICALS & BIOCHEMICALS: NADP; GLUCOSE; GLUCOSE:FRUCTOSE
OXIDOREDUCTASE
MISCELLANEOUS TERMS: Research Article; EC 1.1.99.X; ENZYMOLOGY;
EXPRESSION; GFO GENE; MOLECULAR GENETICS; NADP-CONTAINING
GLUCOSE:FRUCTOSE OXIDOREDUCTASE; PRECURSOR FORMATION; PROCESSING
CONCEPT CODES:
10064 Biochemical Studies-Proteins, Peptides and Amino Acids
10806 Enzymes-Chemical and Physical
31000 Physiology and Biochemistry of Bacteria
31500 Genetics of Bacteria and Viruses
BIOSYSTEMATIC CODES:
06700 Facultatively Anaerobic Gram-Negative Rods (1992-)
06702 Enterobacteriaceae (1992

6/9/31 (Item 31 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

11625814 BIOSIS NO.: 199800408179
Trypanosoma rangeli sialidase: Kinetics of release and antigenic
characterization.
AUTHOR: Saldana Azael; Sousa Octavio E; Orn Anders; Harris Robert A(a)
AUTHOR ADDRESS: (a)Microbiol. Tumorbiol. Cent., Karolinska Inst., Box 280,
S-171 77 Stockholm**Sweden
JOURNAL: Acta Tropica 70 (1):p87-99 June 15, 1998
ISSN: 0001-706X
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The epimastigote stage of Trypanosoma rangeli release a sialidase
with a high sialic acid hydrolysis capacity. We demonstrate that
sialidase secretion is an active process that is reduced at low
temperatures and in the presence of sodium azide . The enzyme is
continuously released until certain maximally active concentrations are
attained in the BHI culture medium when the parasite density reaches 2-3
X 106 cells. When introduced into culture medium already containing such
enzyme levels, freshly harvested parasites do not secrete additional
sialidase. These findings suggest a self-regulating mechanism and a
biological role for the secreted T. rangeli sialidase. The secreted
enzyme was purified to homogeneity by fractionation with ammonium
sulphate and affinity chromatography. Antibodies raised against the
purified molecule recognized antigens of similar molecular weights (73
kDa) in western immunoblotting analyses of T. rangeli and T. cruzi whole
cell lysates. No antigenic recognition was recorded against T. cruzi
active sialidase/trans-sialidase polypeptides or Clostridium perfringens
and Vibrio cholerae commercial sialidases. These observations may
indicate the expression of different antigenic domains in T. rangeli, T.
cruzi and bacterial sialidases.

REGISTRY NUMBERS: 9001-67-6: SIALIDASE; 26628-22-8: SODIUM AZIDE
DESCRIPTORS:
MAJOR CONCEPTS: Parasitology
BIOSYSTEMATIC NAMES: Endospore-forming Gram-Positives--Eubacteria,
Bacteria, Microorganisms; Flagellata--Protozoa, Invertebrata, Animalia;
Vibrionaceae-- Facultatively Anaerobic Gram-Negative Rods,
Eubacteria, Bacteria, Microorganisms
ORGANISMS: Clostridium-perfringens (Endospore-forming Gram-Positives)--
pathogen; Trypanosoma-rangeli (Flagellata)--parasite; Vibrio-cholerae
(Vibrionaceae)--pathogen
BIOSYSTEMATIC CLASSIFICATION (SUPER TAXA): Animals; Bacteria; Eubacteria;
Invertebrates; Microorganisms; Protozoans
CHEMICALS & BIOCHEMICALS: sialidase; sodium azide
CONCEPT CODES:
60502 Parasitology-General
10802 Enzymes-General and Comparative Studies; Coenzymes

BIOSYSTEMATIC CODES:
06704 Vibrionaceae (1992-)
07810 Endospore-forming Gram-Positives (1992-)
35200 Flagellata

6/9/32 (Item 32 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

11621510 BIOSIS NO.: 199800403579
Probing the mechanism of Bacillus 1,3-1,4-beta-D-glucan 4-glucanohydrolases
by chemical rescue of inactive mutants at catalytically essential
residues.
AUTHOR: Viladot Josep-Lluis; Ramon Elisabet De; Durany Olga; Planas Antoni
(a)
AUTHOR ADDRESS: (a)Lab. Biochem., Dep. Org. Chem., Inst. Quimic Sarria,
Univ. Ramon Llull, Via Augusta 390, 08017 B**Spain
JOURNAL: Biochemistry 37 (32):p11332-11342 Aug. 11, 1998
ISSN: 0006-2960
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The role of the key catalytic residues Glu134 and Glu138 in the
retaining 1,3-1,4-beta-glucanase from Bacillus licheniformis is probed by
a chemical rescue methodology based on enzyme activation of inactive
mutants by the action of added nucleophiles. While Glu134 was proposed as
the catalytic nucleophile on the basis of affinity labeling experiments,
no functional proof supported the assignment of Glu138 as the general
acid-base catalyst. Alanine replacements are prepared by site-directed
mutagenesis, to produce the inactive E138A and E134A mutants. Addition of
azide reactivates the mutants in a concentration-dependent manner using
an activated 2,4-dinitrophenyl glycoside substrate. The chemical rescue
operates by a different mechanism depending on the mutant as deduced from
1H NMR monitoring and kinetic analysis of enzyme reactivation. E138A
yields the beta-glycosyl azide product arising from nucleophilic attack
of azide on the glycosyl-enzyme intermediate, thus proving that Glu138
is the general acid-base residue. Azide activates the deglycosylation
step (increasing kcat), but it also has a large effect on a previous step
(as seen by the large decrease in KM, the increase in kcat/KM, and the pH
dependence of activation), probably increasing the rate of glycosylation
through Bronsted acid catalysis by enzyme-bound HN3. By contrast, azide
reactivates the E134A mutant through a single inverting displacement to
give the alpha-glycosyl azide product, consistent with Glu134 being the
catalytic nucleophile. Formate as an exogenous nucleophile has no effect
on the E138A mutant, whereas it is a better activator of E134A than
azide . Although the reaction yields the normal hydrolysis product, a
transient compound was detected by 1H NMR, tentatively assigned to the
alpha-glycosyl formate adduct. This is the first case where a nonmodified
sugar gives a long-lived covalent intermediate that mimics the proposed
glycosyl-enzyme intermediate of retaining glycosidases.
REGISTRY NUMBERS: 9041-22-9: BETA-GLUCAN
DESCRIPTORS:
MAJOR CONCEPTS: Enzymology (Biochemistry and Molecular Biophysics);
Methods and Techniques
BIOSYSTEMATIC NAMES: Endospore-forming Gram-Positives--Eubacteria,
Bacteria, Microorganisms; Enterobacteriaceae--Facultatively
Anaerobic Gram-Negative Rods, Eubacteria, Bacteria, Microorganisms
ORGANISMS: Bacillus-licheniformis (Endospore-forming Gram-Positives);
Escherichia-coli (Enterobacteriaceae)--expression system, strain-TG1
BIOSYSTEMATIC CLASSIFICATION (SUPER TAXA): Bacteria; Eubacteria;
Microorganisms
CHEMICALS & BIOCHEMICALS: barley beta-glucan; pUC119--expression
vector; Bacillus 1,3-1,4-beta-D-glucan 4-glucanhydrolases--expression,
purification; CM-Sepharose--Pharmacia Biotech
METHODS & EQUIPMENT: cation exchange chromatography--column
chromatography, purification method; fast protein liquid
chromatography--liquid chromatography, purification method;
site-directed mutagenesis--molecular genetic method, mutagenesis,
protein engineering; Cary 4E spectrophotometer--laboratory equipment;
NMR--analytical method, spectroscopic techniques--CB; PCR {polymerase
chain reaction}--DNA amplification, mutagenesis, amplification method;
SDS-PAGE {SDS-polyacrylamide gel electrophoresis}; UV

spectrophotometry--analytical method, spectrophotometry--CB; Varian
Gemini 300 spectrometer--Varian, laboratory equipment
CONCEPT CODES:
10802 Enzymes-General and Comparative Studies; Coenzymes
10050 Biochemical Methods-General
10060 Biochemical Studies-General
BIOSYSTEMATIC CODES:
06702 Enterobacteriaceae (1992-)
07810 Endospore-forming Gram-Positives (1992-)

6/9/33 (Item 33 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

11588199 BIOSIS NO.: 199800368895
Evidence against the double-arginine motif as the only determinant for
protein translocation by a novel Sec-independent pathway in Escherichia
coli.
AUTHOR: Brueser Thomas(a); Deutzmann Rainer; Dahl Chrstiane
AUTHOR ADDRESS: (a)Inst. Mikrobiol. Biotechnol., Rheinische
Friedrich-Wilhelms-Univ. Bonn, Meckenhemer Allee 168, D**Germany
JOURNAL: FEMS Microbiology Letters 164 (2):p329-336 July 15, 1998
ISSN: 0378-1097
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Proteins which are synthesized with a signal peptide containing a
'double-arginine' motif may be translocated across the bacterial
cytoplasmic membrane by a mechanism that is different from the known Sec
and signal recognition particle pathways. The function of the
double-arginine motif as a determinant for this novel pathway was studied
by expressions of gene constructs coding for the high potential
iron-sulfur protein (HiPIP) from Chromatium vinosum D in Escherichia
coli. When the protein was produced with its original double-arginine
motif-containing signal peptide, it was in part translocated into the
periplasm and thereby processed, as shown by immunoblots after cell
fractionation and N-terminal sequencing of purified HiPIP. Processing was
not inhibited significantly by 3 mM sodium azide , indicating that
translocation of HiPIP occurs by a SecA-independent pathway.
Translocation of HiPIP could be altered to the SecA-dependent mode when
its signal peptide was substituted by that of PelB from Erwinia
carotovora. When the HiPIP double-arginine motif (SRRDAVK) was introduced
into the corresponding position of the PelB signal peptide, the transport
pathway remained SecA-dependent. This indicates that additional
determinants are required for translocation by the Sec-independent
pathway.

REGISTRY NUMBERS: 74-79-3Q: ARGININE; 7200-25-1Q: ARGININE
DESCRIPTORS:
MAJOR CONCEPTS: Biochemistry and Molecular Biophysics; Membranes (Cell
Biology)
BIOSYSTEMATIC NAMES: Enterobacteriaceae--Facultatively Anaerobic
Gram-Negative Rods, Eubacteria, Bacteria, Microorganisms
ORGANISMS: Escherichia-coli (Enterobacteriaceae)
ORGANISMS: PARTS ETC: bacterial cell membrane
BIOSYSTEMATIC CLASSIFICATION (SUPER TAXA): Bacteria; Eubacteria;
Microorganisms
CHEMICALS & BIOCHEMICALS: protein--translocation; signal peptide--
double arginine motif; Chromatium vinosum high potential iron-sulfur
protein--processing; SecA
CONCEPT CODES:
31000 Physiology and Biochemistry of Bacteria
10064 Biochemical Studies-Proteins, Peptides and Amino Acids
10506 Biophysics-Molecular Properties and Macromolecules
10508 Biophysics-Membrane Phenomena
BIOSYSTEMATIC CODES:
06702 Enterobacteriaceae (1992-)

6/9/34 (Item 34 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

11536178 BIOSIS NO.: 199800317510
Catalase catalyzes of peroxynitrite-mediated phenolic nitration.
AUTHOR: Kono Yasuhisa(a); Yamasaki Tomoaki; Ueda Akane; Shibata Hitoshi
AUTHOR ADDRESS: (a)Dep. Life Sci. Biotechnol., Fac. Life Environmental
Sci., Shimane Univ., Matsue, Shimane 690**Japan
JOURNAL: Bioscience Biotechnology and Biochemistry 62 (3):p448-452 March,
1998
ISSN: 0916-8451

DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Catalase catalyzed the peroxynitrite-mediated nitration of
4-hydroxyphenylacetic acid. The curve for the pH dependence of nitration
was similar to that for the reaction between peroxynitrite and phenol.
Cyanide, azide , and 3-amino-1,2,4-triazole inhibited the nitration in a
dose-dependent way. When catalase was mixed with peroxynitrite, Compound
I was detected as an intermediate. Because azide was an electron donor
for the peroxidatic action of catalase, and because
3-amino-1,2,4-triazole inhibited catalase activity by binding with
Compound I, peroxynitrite-mediated phenolic nitration was probably
accompanied by Compound I formation. Both catalase and superoxide
dismutase protected Escherichia coli from peroxynitrite toxicity.

REGISTRY NUMBERS: 9001-05-2: CATALASE; 19059-14-4: PEROXYNITRITE; 9054-89-1
: SUPEROXIDE DISMUTASE
DESCRIPTORS:
MAJOR CONCEPTS: Enzymology (Biochemistry and Molecular Biophysics)
BIOSYSTEMATIC NAMES: Enterobacteriaceae--Facultatively Anaerobic
Gram-Negative Rods, Eubacteria, Bacteria, Microorganisms
ORGANISMS: Escherichia-coli (Enterobacteriaceae)
BIOSYSTEMATIC CLASSIFICATION (SUPER TAXA): Bacteria; Eubacteria;
Microorganisms
CHEMICALS & BIOCHEMICALS: catalase; superoxide dismutase;
4-hydroxyphenylacetic acid--peroxynitrite-mediated phenolic nitration
CONCEPT CODES:
10802 Enzymes-General and Comparative Studies; Coenzymes
10060 Biochemical Studies-General
30000 Bacteriology, General and Systematic
BIOSYSTEMATIC CODES:
06702 Enterobacteriaceae (1992-)

6/9/35 (Item 35 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

11519670 BIOSIS NO.: 199800301002
The dinuclear center of cytochrome bo3 from Escherichia coli.
AUTHOR: Watmough Nicholas J(a); Cheesman Myles R; Butler Clive S; Little
Richard H; Greenwood Colin; Thomson Andrew J
AUTHOR ADDRESS: (a)Cent. Metalloprotein Spectroscopy Biol., Sch Biol. Sci.,
Univ. East Anglia, Norwich NR4 7TJ**UK
JOURNAL: Journal of Bioenergetics and Biomembranes 30 (1):p55-62 Feb.,
1998
ISSN: 0145-479X
DOCUMENT TYPE: Literature Review
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: For the study of the dinuclear center of heme-copper oxidases
cytochrome bo3 from Escherichia coli offers several advantages over the
extensively characterized bovine cytochrome c oxidase. The availability
of strains with enhanced levels of expression allows purification of the
significant amounts of enzyme required for detailed spectroscopic
studies. Cytochrome bo3 is readily prepared as the fast form, with a
homogeneous dinuclear center which gives rise to characteristic broad EPR
signals not seen in CcO. The absence of CuA and the incorporation of
protohemes allows for a detailed interpretation of the MCD spectra
arising from the dinuclear center heme o3- Careful analysis allows us to
distinguish between small molecules that bind to heme o3, those which are
ligands of CuB, and those which react to yield higher oxidation states of
heme o3. Here we review results from our studies of the reactions of fast
cytochrome bo3 With formate, fluoride, chloride, azide , cyanide, NO2
and H2O2.

REGISTRY NUMBERS: 37256-43-2: NITRIC OXIDE REDUCTASE; 9001-16-5: CYTOCHROME
C OXIDASE
DESCRIPTORS:
MAJOR CONCEPTS: Bioenergetics (Biochemistry and Molecular Biophysics);
Enzymology (Biochemistry and Molecular Biophysics)
BIOSYSTEMATIC NAMES: Enterobacteriaceae--Facultatively Anaerobic
Gram-Negative Rods, Eubacteria, Bacteria, Microorganisms
ORGANISMS: Escherichia-coli (Enterobacteriaceae)
BIOSYSTEMATIC CLASSIFICATION (SUPER TAXA): Bacteria; Eubacteria;
Microorganisms
CHEMICALS & BIOCHEMICALS: cytochrome bo-3--dinuclear center,
heme-copper oxidases; cytochrome c oxidase; nitric oxide

reductase;
quinol oxidase
METHODS & EQUIPMENT: EPR spectroscopy--analytical method; MCD spectroscopy--analytical method
CONCEPT CODES:
10806 Enzymes-Chemical and Physical
10064 Biochemical Studies-Proteins, Peptides and Amino Acids
10065 Biochemical Studies-Porphyrins and Bile Pigments
10506 Biophysics-Molecular Properties and Macromolecules
10510 Biophysics-Bioenergetics: Electron Transport and Oxidative Phosphorylation
10802 Enzymes-General and Comparative Studies; Coenzymes
31000 Physiology and Biochemistry of Bacteria
BIOSYSTEMATIC CODES:
06702 Enterobacteriaceae (1992-)

6/9/36 (Item 36 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

11436289 BIOSIS NO.: 199800217621
Identification of a sequence motif that confers SecB dependence on a SecB-independent secretory protein in vivo.
AUTHOR: Kim Jinoh; Kendall Debra A(a)
AUTHOR ADDRESS: (a)Dep. Molecular Cell Biol., Box U-44, Univ. Connecticut, Storrs, CT 06269**USA
JOURNAL: Journal of Bacteriology 180 (6):p1396-1401 March, 1998
ISSN: 0021-9193
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: SecB is a cytosolic chaperone which facilitates the transport of a subset of proteins, including membrane proteins such as PhoE and LamB and some periplasmic proteins such as maltose-binding protein, in Escherichia coli. However, not all proteins require SecB for transport, and proteins such as ribose-binding protein are exported efficiently even in SecB-null strains. The characteristics which confer SecB dependence on some proteins but not others have not been defined. To determine the sequence characteristics that are responsible for the SecB requirement, we have inserted a systematic series of short, polymeric sequences into the SecB-independent protein alkaline phosphatase (PhoA). The extent to which these simple sequences convert alkaline phosphatase into a SecB-requiring protein was evaluated in vivo. Using this approach we have examined the roles of the polarity and charge of the sequence, as well as its location within the mature region, in conferring SecB dependence. We find that an insert with as few as 10 residues, of which 3 are basic, confers SecB dependence and that the mutant protein is efficiently exported in the presence of SecB. Remarkably, the basic motifs caused the protein to be translocated in a strict membrane potential-dependent fashion, indicating that the membrane potential is not a barrier to, but rather a requirement for, translocation of the motif. The alkaline phosphatase mutants most sensitive to the loss of SecB are those most sensitive to inhibition of SecA via azide treatment, consistent with the necessity for formation of a preprotein-SecB-SecA complex.
Furthermore, the impact of the basic motif depends on location within the mature protein and parallels the accessibility of the location to the secretion apparatus.

REGISTRY NUMBERS: 9001-78-9: ALKALINE PHOSPHATASE
DESCRIPTORS:
MAJOR CONCEPTS: Biochemistry and Molecular Biophysics; Membranes (Cell Biology)
BIOSYSTEMATIC NAMES: Enterobacteriaceae--Facultatively Anaerobic Gram-Negative Rods, Eubacteria, Bacteria, Microorganisms
ORGANISMS: Escherichia-coli (Enterobacteriaceae)
BIOSYSTEMATIC CLASSIFICATION (SUPER TAXA): Bacteria; Eubacteria; Microorganisms
CHEMICALS & BIOCHEMICALS: alkaline phosphatase; SecB--cytosolic protein, molecular chaperone
MISCELLANEOUS TERMS: sequence motif identification; SecB-dependent protein transport
CONCEPT CODES:
31000 Physiology and Biochemistry of Bacteria
10064 Biochemical Studies-Proteins, Peptides and Amino Acids
10506 Biophysics-Molecular Properties and Macromolecules
10508 Biophysics-Membrane Phenomena
BIOSYSTEMATIC CODES:
06702 Enterobacteriaceae (1992-)

6/9/37 (Item 37 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

11340458 BIOSIS NO.: 199800121790
Development of a spectrophotometric immunoagglutination assay for
quantitation of IgG for Escherichia coli 0157.
AUTHOR: Abolmaaty A; Levin R E(a); Abdallah M A
AUTHOR ADDRESS: (a)Dep. Food Sci., Massachusetts Agric. Exp. Stn., Univ.
Massachusetts, Amherst, MA 01003**USA
JOURNAL: Microbios 91 (366):p37-46 1997
ISSN: 0026-2633
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: A direct spectrophotometric immuno-agglutination assay for
quantitation of specific Escherichia coli O157 IgG was developed. Initial
linear rates of increase in absorbance at 550 nm as a result of
agglutination were found to increase with both cell and antiserum
concentrations. Optimum conditions consisted of 1 X 108 cells/ml,
40degree C, and 0.005 M phosphate buffer (PB) containing 0.05% NaCl and
0.02% sodium azide at pH 7.4. A completely linear increase in
absorbance was obtained with affinity purified IgG under optimum
conditions of the assay. The useful range of the assay was between 13 and
104 mug of O157 specific IgG per ml of reaction mixture.

DESCRIPTORS:
MAJOR CONCEPTS: Immune System (Chemical Coordination and Homeostasis);
Infection; Methods and Techniques
BIOSYSTEMATIC NAMES: Enterobacteriaceae--Facultatively Anaerobic
Gram-Negative Rods, Eubacteria, Bacteria, Microorganisms; Leporidae--
Lagomorpha, Mammalia, Vertebrata, Chordata, Animalia
ORGANISMS: rabbit (Leporidae)--host; Escherichia-coli
(Enterobacteriaceae)--serovar-O157:H7, strain-C9490
BIOSYSTEMATIC CLASSIFICATION (SUPER TAXA): Animals; Bacteria; Chordates;
Eubacteria; Lagomorphs; Mammals; Microorganisms; Nonhuman Mammals;
Nonhuman Vertebrates; Vertebrates
CHEMICALS & BIOCHEMICALS: antigen-antibody complex; IgG {immunoglobulin
G}
METHODS & EQUIPMENT: spectrophotometric immunoagglutination assay--
analytical method, antibody detection method

CONCEPT CODES:
34502 Immunology and Immunochemistry-General; Methods
10064 Biochemical Studies-Proteins, Peptides and Amino Acids
10504 Biophysics-General Biophysical Techniques
32000 Microbiological Apparatus, Methods and Media
34504 Immunology and Immunochemistry-Bacterial, Viral and Fungal
36002 Medical and Clinical Microbiology-Bacteriology
BIOSYSTEMATIC CODES:
06702 Enterobacteriaceae (1992-)
86040 Leporidae

6/9/38 (Item 38 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

11334496 BIOSIS NO.: 199800115828
A novel Sec-independent periplasmic protein translocation pathway in
Escherichia coli.
AUTHOR: Santini Claire-Lise; Ize Berengere; Chanal Angelique; Mueller
Matthias; Giordano Gerard; Wu Long-Fei(a)
AUTHOR ADDRESS: (a)Lab. Chim. Bacterienne, UPR9043 CNRS, Inst. Biol.
Structurale Microbiol., 31 Chemin Joseph Aigui**France
JOURNAL: EMBO (European Molecular Biology Organization) Journal 17 (1):p
101-112 Jan. 2, 1998
ISSN: 0261-4189
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The trimethylamine N-oxide (TMAO) reductase of Escherichia coli
is a soluble periplasmic molybdoenzyme. The precursor of this enzyme
possesses a cleavable N-terminal signal sequence which contains a
twin-arginine motif. By using various moa, mob and mod mutants defective
in different steps of molybdocofactor biosynthesis, we demonstrate that
acquisition of the molybdocofactor in the cytoplasm is a prerequisite for
the translocation of the TMAO reductase. The activation and translocation
of the TMAO reductase precursor are post-translational processes, and
activation is dissociable from translocation. The export of the TMAO
reductase is driven mainly by the proton motive force, whereas sodium
azide exhibits a limited effect on the export. The most intriguing
observation is that translocation of the TMAO reductase across the

cytoplasmic membrane is independent of the SecY, SecE, SecA and SecB
proteins. Depletion of Ffh, a core component of the signal recognition
particle of E. coli, appears to have a slight effect on the export of the
TMAO reductase. These results strongly suggest that the translocation of
the molybdoenzyme TMAO reductase into the periplasm uses a mechanism
fundamentally different from general protein translocation.

DESCRIPTORS:
MAJOR CONCEPTS: Biochemistry and Molecular Biophysics
BIOSYSTEMATIC NAMES: Enterobacteriaceae--Facultatively Anaerobic
Gram-Negative Rods, Eubacteria, Bacteria, Microorganisms
ORGANISMS: Escherichia-coli (Enterobacteriaceae)
BIOSYSTEMATIC CLASSIFICATION (SUPER TAXA): Bacteria; Eubacteria;
Microorganisms
CHEMICALS & BIOCHEMICALS: molybdocofactor; trimethylamine N-oxide
reductase--translocation
MISCELLANEOUS TERMS: protein translocation; Sec-independent periplasmic
protein translocation pathway
CONCEPT CODES:
10060 Biochemical Studies-General
10802 Enzymes-General and Comparative Studies; Coenzymes
BIOSYSTEMATIC CODES:
06702 Enterobacteriaceae (1992-)

6/9/39 (Item 39 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

11302211 BIOSIS NO.: 199800083543
The catalytic cycle of the Escherichia coli SecA ATPase comprises two
distinct preprotein translocation events.
AUTHOR: van Der Wolk Jeroen P W; De Wit Janny G; Driessen Arnold J M(a)
AUTHOR ADDRESS: (a)Dep. Microbiol., Groningen Biomolecular Sci. Biotechnol.
Inst., Univ. Groningen, Kerklaan 30, 97**Netherlands
JOURNAL: EMBO (European Molecular Biology Organization) Journal 16 (24):p
7297-7304 Dec. 15, 1997
ISSN: 0261-4189
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: SecA is the ATP-dependent force generator in the Escherichia coli
precursor protein translocation cascade, and is bound at the membrane
surface to the integral membrane domain of the preprotein translocase.
Preproteins are thought to be translocated in a stepwise manner by
nucleotide-dependent cycles of SecA membrane insertion and de-insertion,
or as large polypeptide segments by the protonmotive force (DELTAp) in
the absence of SecA. To determine the step size of a complete ATP- and
SecA-dependent catalytic cycle, translocation intermediates of the
preprotein proOmpA were generated at limiting SecA translocation ATPase
activity. Distinct intermediates were formed, spaced by intervals of
apprx 5 kDa. Inhibition of the SecA ATPase by azide trapped SecA in a
membrane-inserted state and shifted the step size to 2-2.5 kDa. The
latter corresponds to the translocation elicited by binding of
non-hydrolyzable ATP analogues to SecA, or by the re-binding of partially
translocated polypeptide chains by SecA. Therefore, a complete catalytic
cycle of the preprotein translocase permits the stepwise translocation of
5 kDa polypeptide segments by two consecutive events, i.e. apprx 2.5 kDa
upon binding of the polypeptide by SecA, and another 2.5 kDa upon binding
of ATP to SecA.

REGISTRY NUMBERS: 9000-83-3: ATPASE; 56-65-5Q: ATP; 42530-29-0Q: ATP;
94587-45-8Q: ATP; 111839-44-2Q: ATP
DESCRIPTORS:
MAJOR CONCEPTS: Bioenergetics (Biochemistry and Molecular Biophysics);
Enzymology (Biochemistry and Molecular Biophysics); Membranes (Cell
Biology)
BIOSYSTEMATIC NAMES: Enterobacteriaceae--Facultatively Anaerobic
Gram-Negative Rods, Eubacteria, Bacteria, Microorganisms
ORGANISMS: Escherichia-coli (Enterobacteriaceae)
BIOSYSTEMATIC CLASSIFICATION (SUPER TAXA): Bacteria; Eubacteria;
Microorganisms
CHEMICALS & BIOCHEMICALS: proOmpA--preprotein; SecA ATPase--
ATP-dependent force generator, catalytic cycle, membrane de-insertion,
membrane insertion
MISCELLANEOUS TERMS: energetics; preprotein translocation events;
proton-motive force
CONCEPT CODES:
31000 Physiology and Biochemistry of Bacteria

10064 Biochemical Studies-Proteins, Peptides and Amino Acids
10506 Biophysics-Molecular Properties and Macromolecules
10508 Biophysics-Membrane Phenomena
10510 Biophysics-Bioenergetics: Electron Transport and Oxidative Phosphorylation
10806 Enzymes-Chemical and Physical
13003 Metabolism-Energy and Respiratory Metabolism
13012 Metabolism-Proteins, Peptides and Amino Acids
BIOSYSTEMATIC CODES:
06702 Enterobacteriaceae (1992-)

6/9/40 (Item 40 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

11295087 BIOSIS NO.: 199800076419
Photocleavage of plasmid DNA by the porphyrin meso-tetrakis (4-(carboxymethyleneoxy)phenyl) porphyrin.
AUTHOR: Chatterjee Shampa R; Shetty S J; Devasagayam T P A; Srivastava T S (a)
AUTHOR ADDRESS: (a)Dep. Chem., Indian Inst. Technol., Bombay, Powai, Mumbai 400 076**India
JOURNAL: Journal of Photochemistry and Photobiology B Biology 41 (1-2):p 128-135 Nov., 1997
ISSN: 1011-1344
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT:
meso-Tetrakis(4-(carboxymethyleneoxy)phenyl)porphyrin (H2T4CPP) cleaves pBR322 plasmid DNA to single strand breaks in the presence of molecular oxygen and visible light. The above photocleavage was much more efficient in D2O buffer of sodium phosphate (pD = 7.4) than H2O buffer of sodium phosphate (pH = 7.4). In addition this photocleavage of plasmid DNA was inhibited in the presence of sodium azide , lipoic acid, tert-butanol or mannitol suggesting the involvement of 1O2 and .OH in the photocleavage of plasmid DNA. The photocleavage was observed to be more efficient in the presence of H2T4CPP than in the presence of H2CPP (meso-tetrakis(4-carboxyphenyl)porphyrin). Our spectral studies using UV-visible, fluorescence and circular dichroism techniques suggest that H2T4CPP binds to DNA while H2CPP does not. Thus, the difference in photocleavage may be caused by the nonbinding of H2CPP and by the binding of H2T4CPP to calf thymus (CT) DNA.

DESCRIPTORS:
MAJOR CONCEPTS: Molecular Genetics (Biochemistry and Molecular Biophysics)
BIOSYSTEMATIC NAMES: Enterobacteriaceae--Facultatively Anaerobic Gram-Negative Rods, Eubacteria, Bacteria, Microorganisms
ORGANISMS: Escherichia-coli (Enterobacteriaceae)
BIOSYSTEMATIC CLASSIFICATION (SUPER TAXA): Bacteria; Eubacteria; Microorganisms
CHEMICALS & BIOCHEMICALS: meso-tetrakis {4-(carboxymethyleneoxy)phenyl} porphyrin--photosensitizer; pBR322 plasmid DNA--photocleavage, single strand breaks
CONCEPT CODES:
31500 Genetics of Bacteria and Viruses
06506 Radiation-Radiation Effects and Protective Measures
10062 Biochemical Studies-Nucleic Acids, Purines and Pyrimidines
10065 Biochemical Studies-Porphyrins and Bile Pigments
10506 Biophysics-Molecular Properties and Macromolecules
10604 External Effects-Light and Darkness
31000 Physiology and Biochemistry of Bacteria
BIOSYSTEMATIC CODES:
06702 Enterobacteriaceae (1992-)

6/9/41 (Item 41 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

11223057 BIOSIS NO.: 199800004389
Glutamate-286 mutants of cytochrome bo-type ubiquinol oxidase from Escherichia coli: Influence of mutations on the binuclear center structure revealed by FT-IR and EPR spectroscopies.
AUTHOR: Tsubaki Motonari(a); Hori Hiroshi; Mogi Tatsushi
AUTHOR ADDRESS: (a)Dep. Life Sci., Fac. Sci., Himeji Inst. Technol., Kamigoori-cho, Akou-gun, Hyogo 678-12**Japan
JOURNAL: FEBS Letters 416 (3):p247-250 Oct. 27, 1997
ISSN: 0014-5793
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Glutamate-286 mutants of cytochrome bo-type ubiquinol oxidase from Escherichia coli were examined by EPR and FT-IR

spectroscopies. We
confirmed a very low enzymatic activity for E286Q. However, E286D
retained one-third of the wild-type activity, probably due to the
presence of the carboxylic group on the side-chain. The effect of the
mutations at position 286 on the binuclear site was observed clearly in
the EPR spectral change for the air-oxidized state. The effect was more
significantly manifested in the presence of cyanide or azide in the
oxidized state. In contrast, the mutations only slightly perturbed the
binuclear center of the CO-reduced enzymes. These results indicate the
importance of a direct through-bond connectivity between CuB and Glu286
via Pro285 and His284.

REGISTRY NUMBERS: 69671-26-7: UBIQUINOL OXIDASE
DESCRIPTORS:
MAJOR CONCEPTS: Enzymology (Biochemistry and Molecular Biophysics);
Methods and Techniques; Molecular Genetics (Biochemistry and Molecular
Biophysics)
BIOSYSTEMATIC NAMES: Enterobacteriaceae--Facultatively Anaerobic
Gram-Negative Rods, Eubacteria, Bacteria, Microorganisms
ORGANISMS: Escherichia-coli (Enterobacteriaceae)
BIOSYSTEMATIC CLASSIFICATION (SUPER TAXA): Bacteria; Eubacteria;
Microorganisms
CHEMICALS & BIOCHEMICALS: cytochrome bo-type ubiquinol oxidase--
glutamate-286 mutant
METHODS & EQUIPMENT: EPR spectroscopy--analytical method; FT-IR
spectroscopy {Fourier transform IR spectroscopy}--analytical method
CONCEPT CODES:
10806 Enzymes-Chemical and Physical
10508 Biophysics-Membrane Phenomena
31000 Physiology and Biochemistry of Bacteria
31500 Genetics of Bacteria and Viruses
10064 Biochemical Studies-Proteins, Peptides and Amino Acids
BIOSYSTEMATIC CODES:
06702 Enterobacteriaceae (1992-)

6/9/42 (Item 42 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

10833774 BIOSIS NO.: 199799454919
Expression of the Zymomonas mobilis gfo gene for NADP-containing glucose:
Fructose oxidoreductase (GFOR) in Escherichia coli: Formation of
enzymatically active preGFOR but lack of processing into a stable
periplasmic protein.
AUTHOR: Wiegert Thomas; Sahm Hermann; Sprenger Georg A(a)
AUTHOR ADDRESS: (a)Inst. Biotechnol. 1, Forschungszentrum Juelich GmbH,
Postfach 1913, D-52425 Juelich**Germany
JOURNAL: European Journal of Biochemistry 244 (1):p107-112 1997
ISSN: 0014-2956
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Glucose:fructose oxidoreductase (GFOR) of the gram-negative
bacterium Zymomonas mobilis is a periplasmic enzyme with tightly bound
cofactor NADR The preprotein carries an unusually long Nterminal signal
peptide of 52 amino acid residues. Expression of the gfo gene in cells of
Escherichia coli K12, under the control of a tac promoter, led to
immunologically detectable proteins in western blots. and to the
formation of an enzymatically active precursor form (preGFOR), located in
the cytosol. Processing of preGFOR to the mature form was not observed in
E. coli. Replacement of the authentic GFOR signal peptide by the shorter
signal peptides of PhoA or OmpA from E. coli led to processing of the
respective GFOR precursor proteins. However. the processed proteins were
unstable and rapidly degraded in the periplasm unless an E. coli mutant
was used that carried a triple lesion for periplasmic and outer-membrane
proteases. When fusion-protein export was inhibited by sodium azide or
carboxylcyanide mchlorophenylhydrazone, the cytoplasmic precursor forms
of the respective preGFOR were not degraded. A major protease-resistant
GFOR peptide from the OmpA-GFOR fusion was found within spheroplasts of
E. coli to which NADP had been added externally. The formation of this
peptide did not occur in the presence of NAD. It is concluded that NADP
is required for GFOR to fold into its native conformation and that its
absence from the E. coli periplasm is responsible for failure to form a
stable periplasmic protein. The results strongly suggest that, in Z.

mobilis, additional protein factors are required for the transport of
NADP across the plasma membrane and/or incorporation of NADP into the
GFOR apoenzyme.

REGISTRY NUMBERS: 53-59-8: NADP; 50-99-7: GLUCOSE; 94949-35-6:
GLUCOSE:FRUCTOSE OXIDOREDUCTASE
DESCRIPTORS:
MAJOR CONCEPTS: Biochemistry and Molecular Biophysics; Enzymology
(Biochemistry and Molecular Biophysics); Genetics; Physiology
BIOSYSTEMATIC NAMES: Enterobacteriaceae--Eubacteria, Bacteria;
Facultatively Anaerobic Gram-Negative Rods--Eubacteria, Bacteria
ORGANISMS: facultatively anaerobic gram-negative rods (Facultatively
Anaerobic Gram-Negative Rods); Escherichia coli (Enterobacteriaceae)
; Zymomonas mobilis (Facultatively Anaerobic Gram-Negative Rods)
BIOSYSTEMATIC CLASSIFICATION (SUPER TAXA): bacteria; eubacteria;
microorganisms
CHEMICALS & BIOCHEMICALS: NADP; GLUCOSE; GLUCOSE:FRUCTOSE
OXIDOREDUCTASE
MISCELLANEOUS TERMS: Research Article; EC 1.1.99.X; ENZYMOLOGY;
EXPRESSION; GFO GENE; MOLECULAR GENETICS; NADP-CONTAINING
GLUCOSE:FRUCTOSE OXIDOREDUCTASE; PRECURSOR FORMATION; PROCESSING
CONCEPT CODES:
10064 Biochemical Studies-Proteins, Peptides and Amino Acids
10806 Enzymes-Chemical and Physical
31000 Physiology and Biochemistry of Bacteria
31500 Genetics of Bacteria and Viruses
BIOSYSTEMATIC CODES:
06700 Facultatively Anaerobic Gram-Negative Rods (1992-)
06702 Enterobacteriaceae (1992

6/9/43 (Item 43 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

10569289 BIOSIS NO.: 199699190434
Export of the periplasmic NADP-containing glucose-fructose oxidoreductase
of Zymomonas mobilis.
AUTHOR: Wiegert Thomas; Sahm Hermann; Sprenger Georg A(a)
AUTHOR ADDRESS: (a)Inst. Biotechnologie 1, Forschungszentrum Juelich GmbH,
Postfach 1913, D-52425 Juelich**Germany

JOURNAL: Archives of Microbiology 166 (1):p32-41 1996
ISSN: 0302-8933
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Glucose-fructose oxidoreductase (GFOR) of the gram-negative
bacterium Zymomonas mobilis is a periplasmic enzyme with the tightly
bound cofactor NADP. The preprotein carries an unusually long N-terminal
signal sequence of 52 amino acid residues. A sorbitol-negative mutant
strain (ACM3963) was found to be deficient in GFOR activity and was used
for the expression of plasmid-borne copies of the wild-type gfo gene or
of alleles encoding alterations in the signal sequence of the preGFOR
protein. Z. mobilis cells with the wild-type gfo allele translocated
pre-GFOR, at least partially, via the Sec pathway since CCCP
(carboxylcyanide-m-chlorophenylhydrazone; uncoupler of proton motive
force) or sodium azide (inhibitor of SecA) abolished the processing of
GFOR. A gfo allele with the hydrophobic region of the signal sequence
removed (residues 32-46; DELTA-32-46) led to a protein that was no longer
processed, but showed full enzymatic activity (180 U/mg) and had the
cofactor NADP firmly bound. A deletion in the n-region of the signal
sequence (residues 2-20; DELTA-2-20) or exchange of the entire GFOR
signal sequence with the signal sequence of gluconolactonase of Z.
mobilis led to active and processed GFOR. Strain ACM3963 could not grow
in the presence of high sugar concentrations (1 M sucrose) unless
sorbitol was added. The presence of the plasmid-borne gfo wild-type
allele or of the DELTA-2-20 deletion led to the restoration of growth on
media with 1 M sucrose, whereas the presence of the DELTA-32-46 deletion
led to a growth behavior similar to that of strain ACM3963, with no
sorbitol formation from sucrose.

REGISTRY NUMBERS: 53-59-8: NADP; 94949-35-6: GLUCOSE-FRUCTOSE
OXIDOREDUCTASE; 50-70-4: SORBITOL
DESCRIPTORS:
MAJOR CONCEPTS: Biochemistry and Molecular Biophysics; Cell Biology;
Enzymology (Biochemistry and Molecular Biophysics);

Genetics; Membranes
(Cell Biology); Metabolism; Methods and Techniques; Molecular Genetics
(Biochemistry and Molecular Biophysics); Nutrition; Physiology
BIOSYSTEMATIC NAMES: Facultatively Anaerobic Gram-Negative Rods--
Eubacteria, Bacteria
ORGANISMS: facultatively anaerobic gram-negative rods (Facultatively
Anaerobic Gram-Negative Rods); Zymomonas mobilis (Facultatively
Anaerobic Gram-Negative Rods)
BIOSYSTEMATIC CLASSIFICATION (SUPER TAXA): bacteria; eubacteria;
microorganisms
CHEMICALS & BIOCHEMICALS: NADP; GLUCOSE-FRUCTOSE OXIDOREDUCTASE;
SORBITOL
MISCELLANEOUS TERMS: DELETIONS; ENZYMOLOGY; GFO-DEFICIENT MUTANT;
GROWTH BEHAVIOR; METABOLISM; MOLECULAR GENETICS; PERIPLASMIC
NADP-CONTAINING GLUCOSE-FRUCTOSE OXIDOREDUCTASE EXPORT; PROTEIN EXPORT;
SIGNAL SEQUENCE; SORBITOL FORMATION; WILD-TYPE GENE
CONCEPT CODES:
10062 Biochemical Studies-Nucleic Acids, Purines and Pyrimidines
10064 Biochemical Studies-Proteins, Peptides and Amino Acids
10068 Biochemical Studies-Carbohydrates
10300 Replication, Transcription, Translation
10504 Biophysics-General Biophysical Techniques
10506 Biophysics-Molecular Properties and Macromolecules
10508 Biophysics-Membrane Phenomena
10806 Enzymes-Chemical and Physical
10808 Enzymes-Physiological Studies
13002 Metabolism-General Metabolism; Metabolic Pathways
13003 Metabolism-Energy and Respiratory Metabolism
13004 Metabolism-Carbohydrates
13012 Metabolism-Proteins, Peptides and Amino Acids
13202 Nutrition-General Studies, Nutritional Status and Methods
30500 Morphology and Cytology of Bacteria
31000 Physiology and Biochemistry of Bacteria
31500 Genetics of Bacteria and Viruses
BIOSYSTEMATIC CODES:
06700 Facultatively Anaerobic Gram-Negative Rods (1992-)

6/9/44 (Item 44 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

07343778 BIOSIS NO.: 000090123680
CHARACTERIZATION OF A GRAM-POSITIVE BACTERIUM FROM THE PROVENTRICULUS OF BUDGERIGARS MELOPSITTACUS-UNDULATUS
AUTHOR: SCANLAN C M; GRAHAM D L
AUTHOR ADDRESS: DEP. VETERINARY MICROBIOLOGY PARASITOLOGY, TEXAS VETERINARY
MED. CENTER, TEXAS A AND M UNIVERSITY, COLLEGE STATION, TEXAS 77843-4467.
JOURNAL: AVIAN DIS 34 (3). 1990. 779-786. 1990
FULL JOURNAL NAME: Avian Diseases
CODEN: AVDIA
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: The cellular, cultural, and biochemical characteristics of eight
isolates of a large gram-positive bacillus that are commonly observed as
apparently normal flora in the proventriculus of budgerigars (Melopsittacus undulatus) were determined. The bacterium was highly
pleomorphic and changed markedly in both diameter and length when
subcultured on agar media. The bacterium was facultative anaerobic
and capnophilic, hemolytic on blood agar, and formed flat colonies with
irregular edges after incubation for several days. All isolates grew on
sodium azide agar but did not grow on MacConkey agar. The isolates were
catalase-negative and oxidase-negative and did not reduce nitrate. All
isolates failed to utilize arginine lysine, ornithine or tryptophane but
produced acid from glucose, galactose, levulose, maltose, melibiose,
starch, and sucrose. All isolates produced acetoin from glucose and
hydrolyzed esculin. The eight isolates could not be identified to either
genus or species level based on the descriptions of currently organisms
in the division Firmicutes as described in Bergey's Manual of Systematic
Bacteriology.

CONCEPT CODES:
00504 General Biology-Taxonomy, Nomenclature and Terminology
30000 Bacteriology, General and Systematic
31000 Physiology and Biochemistry of Bacteria
36002 Medical and Clinical Microbiology-Bacteriology
38004 Veterinary Science-Pathology
38006 Veterinary Science-Microbiology
32000 Microbiological Apparatus, Methods and Media
BIOSYSTEMATIC CODES:
04000 Bacteria-Unspecified (1979-)
85558 Psittaciformes
BIOSYSTEMATIC CLASSIFICATION (SUPER TAXA):
Microorganisms

Bacteria
Animals
Chordates
Vertebrates
Nonhuman Vertebrates
Birds

6/9/45 (Item 45 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

05581313 BIOSIS NO.: 000083054453
STUDIES ON IMMUNOGENICITY OF PASTEURELLA-MULTOCIDA ISOLATED FROM SWINE IN KOREA
AUTHOR: KIM J Y; PARK J M; KIM O N
AUTHOR ADDRESS: VET. RES. INST., ANYANG, KOREA.
JOURNAL: RES REP RURAL DEV ADM (SUWEON) 28 (LIVEST. AND VET.). 1986. 77-93.
1986
FULL JOURNAL NAME: Research Reports of the Rural Development Administration (Suweon)
CODEN: NSYNE
RECORD TYPE: Abstract
LANGUAGE: KOREAN

ABSTRACT: Pasteurella multocida plays an important role in inducing respiratory disease of pigs. This acts not only as a primary invading organism during the early stages of rearing period but also as a secondary invader to primary organisms such as Bordetella bronchiseptica, Haemophilus spp., or Mycoplasma spp. In this study, isolation and identification of P. multocida were attempted from lung samples and nasal swabs from pigs. Serotyping was performed against capsular and somatic antigen on the isolates and also immunogenicity of P. multocida isolated from pneumonic pigs was tested to develop a vaccine against P. multocida.

The results obtained are as follows. A total of 127 (23.7%) P. multocida were isolated from 536 specimens collected from slaughtered pigs and piglets showing respiratory signs. Of 127 P. multocida, 95 isolates were from 414 cases of pneumonic lungs of slaughtered pigs and 32 from nasal swab specimens of 122 piglets infected with respiratory disease. Capsular serotyping performed on the 127 P. multocida revealed that 47 strains (37.0%) were A type (Carter's) and 38 strains (29.9%) were D type and the remainder were untypable. When serotyping was performed against somatic antigen on the 85 strains capsular types of which were identified as described above 14, 15, 5, 11 and 19 strains belonged to 1A, 3A, 5A, 2D and 4D, respectively. Among antigens prepared by various inactivation methods; heat, formalin, phenol, sodium azide or merthiolate, formalin treated antigen was found to the most immunogenic in mice, i.e. 94 per cent of mice inoculated with the antigen were protected against P. multocida challenged. In the cross immunity test between P. multocida serotype A or D, 79 to 100 per cent were protected against homologous challenge, while 50 to 73 per cent were protected against heterologous challenge. The mouse protection rates of formalin treated antigen containing incomplete freund's adjuvant (IFA), aluminum hydroxide-gel (AHG) or both IFA and AHG were 92, 83 and 84 per cent, respectively.

Divalent antigen containing of serotype A and D protected 97 and 94 per cent of mice when used with adjuvants respectively with AHG and IFA, while the antigen gave 64 per cent without adjuvant. The antigen which induced 93 to 94 per cent protection in mice gave 82 to 87 per cent and 44 to 50 per cent protection when inoculated with 0.56 .times. 109 cells and 0.11 .times. 109 cells of its original, respectively. All the pigs immunized with the experimental antigen were protected from challenge exposure, while 50 to 75 per cent of pigs survived when one fifth of the recommended does was used. When the pigs were inoculated with divalent antigen twice at 55 and 70 days of age, the antibody titers at 3 week post-inoculation were 20 by passive mouse protection (PMP) test and 133 to 160 by indirect haemagglutination (IHA) test. Sows were vaccinated twice with the adjuvanted antigen at 30 to 15 days before paturition and then sera were collected to antibody determination. The antibody titers at paturition were 40 by PMP test and 160 to 320 by IHA test. It was found that antibody titers of colostrum were higher than those of from dams. Passive antibody titers to P. multicoida in piglets were 30 by PMP

and 60 by IHA test at 10 days of age and gradually disappeared as age
increased the dropped under detectable level by 30 days of age.

DESCRIPTORS: BORDETELLA-BRONCHISEPTICA HAEMOPHILUS-SPP MYCOPLASMA-SPP IMMUNIZATION CHALLENGE PROTECTION ANTIBODY RESPONSE
CONCEPT CODES:
13012 Metabolism-Proteins, Peptides and Amino Acids
16006 Respiratory System-Pathology
22018 Pharmacology-Immunological Processes and Allergy
34504 Immunology and Immunochemistry-Bacterial, Viral and Fungal
36002 Medical and Clinical Microbiology-Bacteriology
38006 Veterinary Science-Microbiology
13004 Metabolism-Carbohydrates
16001 Respiratory System-General; Methods
30500 Morphology and Cytology of Bacteria
31000 Physiology and Biochemistry of Bacteria
34502 Immunology and Immunochemistry-General; Methods
38004 Veterinary Science-Pathology
BIOSYSTEMATIC CODES:
04720 Gram-negative Aerobic Rods and Cocci-Uncertain Affiliation (1979-
)
04814 Gram-negative Facultatively Anaerobic Rods-Uncertain
Affiliation (1979-)
09112 Mycoplasmataceae (1979-)
85740 Suidae
BIOSYSTEMATIC CLASSIFICATION (SUPER TAXA):
Microorganisms
Bacteria
Animals
Chordates
Vertebrates
Nonhuman Vertebrates
Mammals
Nonhuman Mammals
Artiodactyls

6/9/46 (Item 46 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

05243315 BIOSIS NO.: 000082083937
OXIDATIVE INACTIVATION OF ACTINOBACILLUS-ACTINOMYCETEMCOMITANS LEUKOTOXIN
BY THE NEUTROPHIL MYELOPEROXIDASE SYSTEM
AUTHOR: CLARK R A; LEIDAL K G; TAICHMAN N S
AUTHOR ADDRESS: DEP. MED., VA MED. CENTER, IOWA CITY, IOWA 52242.
JOURNAL: INFECT IMMUN 53 (2). 1986. 252-256. 1986
FULL JOURNAL NAME: Infection and Immunity
CODEN: INFIB
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: The leukotoxin of Actinobacillus actinomycetemcomitans has been
implicated in the pathogenesis of inflammatory periodntal disease. We
examined a potential mechanism for detoxification of this microbial
product by the neutrophil myeloperoxidase system. Exposure to
myeloperoxidase, H2O2, and a halide resulted in marked inactivation of
leukotoxin, an effect of which required each component of the
myeloperoxidase system. Toxin inactivation was blocked by agents which
inhibit heme enzymes (azide , cyanide) or degrade H2O2 (catalase).
Reagent H2O2 could be replaced by the peroxide-generating enzyme system
glucose oxidase plus glucose. The latter system, in fact, was more potent
than reagent H2O2 in terms of the capacity to inactivate high
concentrations of toxin. Toxin inactivation was complete within 1 to 2
min at 37.degree.C. These observations suggest a possible role for
oxidative inactivation of leukotoxin by secretory products of neutrophils.

DESCRIPTORS: INFLAMMATORY PERIODONTAL DISEASE HYDROGEN PEROXIDE HALIDE GLUCOSE OXIDASE-GLUCOSE SYSTEM
CONCEPT CODES:
10060 Biochemical Studies-General
10069 Biochemical Studies-Minerals
10808 Enzymes-Physiological Studies
15004 Blood, Blood-Forming Organs and Body Fluids-Blood Cell Studies
15008 Blood, Blood-Forming Organs and Body Fluids-Lymphatic Tissue and
Reticuloendothelial System
22501 Toxicology-General; Methods and Experimental
22505 Toxicology-Antidotes and Preventative Toxicology (1972-)
36002 Medical and Clinical Microbiology-Bacteriology
10064 Biochemical Studies-Proteins, Peptides and Amino Acids
10068 Biochemical Studies-Carbohydrates
10804 Enzymes-Methods
12508 Pathology, General and Miscellaneous-Inflammation and
Inflammatory Disease
18006 Bones, Joints, Fasciae, Connective and Adipose Tissue-Pathology
19006 Dental and Oral Biology-Pathology
34504 Immunology and Immunochemistry-Bacterial, Viral and Fungal

34508 Immunology and Immunochemistry-Immunopathology, Tissue Immunology
BIOSYSTEMATIC CODES:
04814 Gram-negative Facultatively Anaerobic Rods-Uncertain
Affiliation (1979-)
BIOSYSTEMATIC CLASSIFICATION (SUPER TAXA):
Microorganisms
Bacteria

6/9/47 (Item 47 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

05135169 BIOSIS NO.: 000081093294
INFLUENCE OF ENDOGENOUS CATALASE ACTIVITY ON THE SENSITIVITY OF THE ORAL BACTERIUM ACTINOBACILLUS-ACTINOMYCETEMCOMITANS AND THE ORAL HAEMOPHILI TO THE BACTERICIDAL PROPERTIES OF HYDROGEN PEROXIDE
AUTHOR: MIYASAKI K T; WILSON M E; ZAMBON J J; GENCO R J
AUTHOR ADDRESS: DEP. ORAL BIOLOGY, STATE UNIV. NEW YORK AT BUFFALO, BUFFALO, NY 14214, USA.
JOURNAL: ARCH ORAL BIOL 30 (11-12). 1985 (RECD. 1986). 843-848. 1985
FULL JOURNAL NAME: Archives of Oral Biology
CODEN: AOBIA
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: Actinobacillus actinomycetemcomitans and the genetically-related oral haemophili (Haemophilus segnis, Haemophilus aprhophilus and Haemophilus paraphrophilus) exhibit a range of sensitivities to the lethal effect of hydrogen peroxide (H2O2), A. actinomycetemcomitans being the most resistant. To extend this information, susceptibility to a range of H2O2 concentrations (10-6-10-3 M) was assessed by incubating bacterial suspensions for 1 h at 37.degree. C in the presence of H2O2 and spreading the suspensions on chocolate agar plates to determine the concentration of H2O2 producing a 50 per cent reduction in colony-forming units (LD50). Catalase activity was quantified with a Clark-type oxygen electrode, which polarographically monitored the formation of dissolved oxygen in bacterial suspensions on sonicates following addition of reagent H2O2. Sensitivity to H2O2 did not correlate with catalase activity, either in intact cells or in bacterial sonicates. Specifically, some bacterial strains with undetectable catalase activity were highly resistant to H2O2. Micromolar concentrations of sodium azide which completely inhibited cell-associated catalase activity did not affect the resistance of A. actinomycetemcomitans to H2O2. Thus, the endogenous catalase activity of A. actinomycetemcomitans and certain oral haemophili is not an important determinant of resistance to the bactericidal effects of H2O2.

DESCRIPTORS: HAEMOPHILUS-SEGNIS
HAEMOPHILUS-APHROPHILUS
HAEMOPHILUS-PARAPHROPHILUS
CONCEPT CODES:
10808 Enzymes-Physiological Studies
13002 Metabolism-General Metabolism; Metabolic Pathways
13012 Metabolism-Proteins, Peptides and Amino Acids
19006 Dental and Oral Biology-Pathology
36002 Medical and Clinical Microbiology-Bacteriology
10060 Biochemical Studies-General
10064 Biochemical Studies-Proteins, Peptides and Amino Acids
19001 Dental and Oral Biology-General; Methods
BIOSYSTEMATIC CODES:
04814 Gram-negative Facultatively Anaerobic Rods-Uncertain
Affiliation (1979-)
BIOSYSTEMATIC CLASSIFICATION (SUPER TAXA):
Microorganisms
Bacteria

6/9/48 (Item 48 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

04353539 BIOSIS NO.: 000078083083
EFFECTS OF METABOLIC INHIBITORS ON THE ALCOHOLIC FERMENTATION BY SEVERAL YEASTS IN BATCH OR IN IMMOBILIZED CELL SYSTEMS
AUTHOR: AMIN G; STANDAERT P; VERACHTERT H
AUTHOR ADDRESS: LAB. INDUSTRIAL MICROBIOL. BIOCHEM., UNIV. LEUVEN, KARDINAAL MERCIERLAAN, 92 B-3030 HEVERLEE-LOUVAIN, BELGIUM.
JOURNAL: APPL MICROBIOL BIOTECHNOL 19 (2). 1984. 91-99. 1984
CODEN: EJABD
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: In previous papers it was shown that the bacterium Zymomonas

mobilis might be an interesting alternative for industrial alcohol
production from sugar, compared to Saccharomyces bayanus. Factors that
might increase the glucose to ethanol conversion efficiency and which are
in favor of the bacterium, are the production of less biomass and less
by-products such as glycerol, succinic acid, butanediol, acetoin and
acetic acid. In order to reduce the synthesis of biomass, 3 metabolic
inhibitors were now studied: dinitrophenol, azide and arsenate. Their
effects on the alcoholic fermentation in batch and in immobilized cell
system were investigated, using 3 yeasts: S. bayanus, Schizosaccharomyces
pombe and S. diastaticus. Dinitrophenol in 0.1 mM concentration was
effective in increasing the conversion of glucose to ethanol especially
with S. bayanus while azide in 0.1 mM concentration was better with S.
pombe. In immobolized systems high steady state ethanol production from
15% glucose media was obtained by inclusion into the media of
dinitrophenol or azide . Arsenate had less effect at the concentrations
used. As a result, ethanol productivity in grams per hour was increased
from around 70 in the absence of inhibitor to around 74 in the presence
of dinitrophenol with S. bayanus. With S. pombe the productivity was
increased from around 65 in the absence of inhibitor to around 74 in the
presence of azide . The specific ethanol productivity expressed as 1 g
ethanol formed per hour and per gram viable cells was increased from 0.87
to 1.37 for S. pombe and from 1.02 to 1.66 for S. bayanus.

DESCRIPTORS: ZYMOMONAS-MOBILIS
SACCHAROMYCES-BAYANUS
SACCHAROMYCES-DIASTATICUS
SCHIZOSACCHAROMYCES-POMBE BIOMASS
ETHANOL
PRODUCTIVITY
CONCEPT CODES:
10511 Biophysics-Bioengineering
13002 Metabolism-General Metabolism; Metabolic Pathways
39007 Food and Industrial Microbiology-Biosynthesis, Bioassay and
Fermentation
51510 Plant Physiology, Biochemistry and Biophysics-Growth,
Differentiation
51519 Plant Physiology, Biochemistry and Biophysics-Metabolism
02504 Cytology and Cytochemistry-Plant
10010 Comparative Biochemistry, General
10050 Biochemical Methods-General
10060 Biochemical Studies-General
10068 Biochemical Studies-Carbohydrates
13003 Metabolism-Energy and Respiratory Metabolism
13004 Metabolism-Carbohydrates
31000 Physiology and Biochemistry of Bacteria
32000 Microbiological Apparatus, Methods and Media
51524 Plant Physiology, Biochemistry and Biophysics-Apparatus and
Methods
BIOSYSTEMATIC CODES:
04814 Gram-negative Facultatively Anaerobic Rods-Uncertain
Affiliation (1979-)
15100 Ascomycetes
BIOSYSTEMATIC CLASSIFICATION (SUPER TAXA):
Microorganisms
Bacteria
Plants
Nonvascular Plants
Fungi

6/9/49 (Item 49 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

04268468 BIOSIS NO.: 000077094514
TEMPERATURE DEPENDENT AZIDE SENSITIVITY OF GROWTH AND ATPASE ACTIVITY IN
THE FACULTATIVE THERMOPHILE BACILLUS-COAGULANS
AUTHOR: JONES M V; SPENCER W N; EDWARDS C
AUTHOR ADDRESS: DEP. OF MICROBIOL., LIFE SCI. BUILDING, UNIV. OF LIVERPOOL,
P.O. BOX 147, LIVERPOOL L69 3BX, UK.
JOURNAL: J GEN MICROBIOL 130 (1). 1984. 95-102. 1984
FULL JOURNAL NAME: Journal of General Microbiology
CODEN: JGMIA
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: Inhibition by sodium azide of the growth of B. coagulans
decreased but the cytochrome content, particularly cytochrome d,
increased with increasing growth temperature. Higher cytochrome d content
correlated with increased cyanide resistance of NADH oxidase, but azide
resistant activity was found in mesophilic and thermophilic cultures.
Anaerobic growth at 37.degree. C was totally inhibited by 1 mM- azide .
At 55.degree. C growth occurred with 4 mM- azide , but the cell yield was
reduced by 60%. ATPase activity was sensitive to azide but

inhibition
varied with both growth and assay temperatures. ATPase from cells grown
at 55.degree. C was least sensitive when assayed at 55.degree. C.
Possible changes in ATPase which could account for the temperature-dependent azide sensitivity are discussed.

DESCRIPTORS: CYTOCHROME D CYANIDE RESISTANCE NADH OXIDASE
CONCEPT CODES:
10614 External Effects-Temperature as a Primary Variable (1971-)
10618 External Effects-Temperature as a Primary Variable-Hot (1971-)
13002 Metabolism-General Metabolism; Metabolic Pathways
13003 Metabolism-Energy and Respiratory Metabolism
31000 Physiology and Biochemistry of Bacteria
10012 Biochemistry-Gases (1970-)
10050 Biochemical Methods-General
10060 Biochemical Studies-General
10064 Biochemical Studies-Proteins, Peptides and Amino Acids
10065 Biochemical Studies-Porphyrins and Bile Pigments
10802 Enzymes-General and Comparative Studies; Coenzymes
10804 Enzymes-Methods
10806 Enzymes-Chemical and Physical
10808 Enzymes-Physiological Studies
22501 Toxicology-General; Methods and Experimental
23001 Temperature: Its Measurement, Effects and Regulation-General
Measurement and Methods
32000 Microbiological Apparatus, Methods and Media
BIOSYSTEMATIC CODES:
05610 Bacillaceae (1979-)
BIOSYSTEMATIC CLASSIFICATION (SUPER TAXA):
Microorganisms
Bacteria

6/9/50 (Item 50 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

03321621 BIOSIS NO.: 000072049725
BACTERIAL SURVIVAL IN A DILUTE ENVIRONMENT
AUTHOR: SJOGREN R E; GIBSON M J
AUTHOR ADDRESS: DEP. MICROBIOL. AND BIOCHEM., UNIV. VERMONT, BURLINGTON, VERMONT 05405.
JOURNAL: APPL ENVIRON MICROBIOL 41 (6). 1981. 1331-1336. 1981
FULL JOURNAL NAME: Applied and Environmental Microbiology
CODEN: AEMID
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: Bacteria were isolated from lake water and their ability to
remain viable in a dilute, nutrient-deficient environment was tested by a
method that permits suspension of test bacteria between 2 appressed
microporous membranes in an aqueous environment. This approach permitted
separation of the lake isolates into 2 categories. Members of the tribe
Klebsielleae had a prolonged survival rate of 40% or better after 24 h;
nonsurvivors were not viable for much longer than 24 h. These
nonsurvivors belonged to the genera Acinetobacter, Aeromonas,
Alcaligenes, Erwinia, Escherichia, Flavobacterium and Pseudomonas.
Differences in RNase and ATPase levels between Escherichia coli
(nonsurvivor) and Klebsiella (survivor) cells were detected. At pH 7.5,
stressed E. coli cells contained 14% of the ATPase activity detected in
the control; at pH 5.5, in the presence of Ca ions, these same cells
contained 50% of the control ATPase levels. At pH 7.2, E. coli cells were
strongly inhibited by an ATPase inhibitor, bathophenanthroline (88%);
oligomycin (64%); and the proton ionophore carbonyl
cyanide-m-chlorophenyl hydrazone (67%). Sodium azide and valinomycin
were only moderately inhibitory (15 and 28%, respectively). Although the
ability to scavenge internal endogenous reserves seems important, certain
enteric bacteria seem capable of using acidic conditions (pH 5.5) as an
electrochemical gradient to generate necessary high-energy intermediates
for prolongation of survival beyond that possible in environments of
near-neutral pH.

DESCRIPTORS: KLEBSIELLEAE ACINETOBACTER AEROMONAS ALCALIGENES ERWINIA ESCHERICHIA FLAVOBACTERIUM PSEUDOMONAS ESCHERICHIA-COLI LAKE WATER PH ATPASE ACTIVITY
CONCEPT CODES:
07514 Ecology; Environmental Biology-Limnology
30000 Bacteriology, General and Systematic
31000 Physiology and Biochemistry of Bacteria
37015 Public Health: Environmental Health-Air, Water and Soil Pollution
07517 Ecology; Environmental Biology-Water Research and Fishery Biology
(1969-1984)
10010 Comparative Biochemistry, General
10050 Biochemical Methods-General

10060 Biochemical Studies-General
10064 Biochemical Studies-Proteins, Peptides and Amino Acids
10502 Biophysics-General Biophysical Studies
10506 Biophysics-Molecular Properties and Macromolecules
10508 Biophysics-Membrane Phenomena
10802 Enzymes-General and Comparative Studies; Coenzymes
10804 Enzymes-Methods
10806 Enzymes-Chemical and Physical
10808 Enzymes-Physiological Studies
13002 Metabolism-General Metabolism; Metabolic Pathways
13003 Metabolism-Energy and Respiratory Metabolism
13012 Metabolism-Proteins, Peptides and Amino Acids
13014 Metabolism-Nucleic Acids, Purines and Pyrimidines
13202 Nutrition-General Studies, Nutritional Status and Methods
13203 Nutrition-Malnutrition; Obesity
22003 Pharmacology-Drug Metabolism; Metabolic Stimulators
32000 Microbiological Apparatus, Methods and Media
37400 Public Health: Microbiology
38502 Chemotherapy-General; Methods; Metabolism
BIOSYSTEMATIC CODES:
04000 Bacteria-Unspecified (1979-)
04716 Pseudomonadaceae (1979-)
04720 Gram-negative Aerobic Rods and Cocci-Uncertain Affiliation (1979-)
04810 Enterobacteriaceae (1979-)
04812 Vibrionaceae (1979-)
04814 Gram-negative Facultatively Anaerobic Rods-Uncertain Affiliation (1979-)
05110 Neisseriaceae (1979-)
BIOSYSTEMATIC CLASSIFICATION (SUPER TAXA):
Microorganisms
Bacteria

6/9/51 (Item 51 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

03200552 BIOSIS NO.: 000071013663
EFFECTS OF CHEMICAL AND HEAT TREATMENTS ON ETHYLENE PRODUCTION IN SOIL
AUTHOR: SUTHERLAND J B; COOK R J
AUTHOR ADDRESS: DEP. BACTERIOL. BIOCHEM., UNIV. IDAHO, MOSCOW, IDAHO 83843, USA.
JOURNAL: SOIL BIOL BIOCHEM 12 (4). 1980. 357-362. 1980
FULL JOURNAL NAME: Soil Biology and Biochemistry
CODEN: SBIOA
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: Factors influencing C2H4 production in a silt loam were investigated to determine the source of this gas in soil. Air-dried samples of soil in glass vials were moistened to .apprx. -10kPa, sealed with rubber septa, and incubated at 30 or 35.degree. C, with an original atmosphere of air or O2-free N2. C2H4 concentrations in the vials were determined by gas chromatography. Addition of the antibacterial agents, chloramphenicol or novobiocin to the soil inhibited C2H4 production, whereas the antifungal agent cycloheximide had no effect. Sodium azide and sodium cyanide also reduced C2H4 production. Treatment of the soil with moist heat (i.e., passing a steam-air mixture through it) at 80.degree. C for 30 min failed to reduce the ability of the soil to produce C2H4 during subsequent incubation at 30.degree. C, but autoclaving it twice at 121.degree. C prevented C2H4 production. As with nonheated soil, C2H4 production from soil treated at 80.degree. C was prevented by novobiocin but not by cycloheximide. Only .apprx. 10% of the bacteria isolated from nontreated soil were spore-formers. In contrast, 95-98% and possibly more of the bacteria isolated from heat-treated soil were spore-formers, including those in soil which was heat-treated and then incubated moist at 30.degree. C for an additional 3 days before dilution plating. Addition of methionine had no effect on the production of C2H4 in anaerobic soil, whereas ethionine, chlorogenic acid and EDTA all enhanced C2H4 production. Ethionine, but not chlorogenic acid or EDTA, also resulted in considerable C2H4 accumulation in autoclaved soil; the C2H4 detected in ethionine-amended soil was apparently nonmicrobial in origin. Soil samples incubated at constant temperatures of 30, 50 or 70.degree. C all produced C2H4. The results collectively indicate that C2H4 in soil is most likely produced by facultative or strictly anaerobic bacteria, which are probably spore-formers and may also be thermophilic. Several isolates of spore-forming bacteria were inoculated into autoclaved soil, but none produced appreciable amounts of C2H4 under the test conditions.
DESCRIPTORS: BACTERIA CHLORAMPHENICOL

NOVOBIOCIN CYCLO HEXIMIDE AUTOCLAVING
GAS CHROMATOGRAPHY
CONCEPT CODES:
10012 Biochemistry-Gases (1970-)
31000 Physiology and Biochemistry of Bacteria
40000 Soil Microbiology
52805 Soil Science-Physics and Chemistry (1970-)
10060 Biochemical Studies-General
10064 Biochemical Studies-Proteins, Peptides and Amino Acids
10504 Biophysics-General Biophysical Techniques
10618 External Effects-Temperature as a Primary Variable-Hot (1971-)
10620 External Effects-Humidity (1972-)
13002 Metabolism-General Metabolism; Metabolic Pathways
22002 Pharmacology-General
38504 Chemotherapy-Antibacterial Agents
38508 Chemotherapy-Antifungal Agents
BIOSYSTEMATIC CODES:
04000 Bacteria-Unspecified (1979-)
BIOSYSTEMATIC CLASSIFICATION (SUPER TAXA):
Microorganisms
Bacteria

6/9/52 (Item 52 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

01943585 BIOSIS NO.: 000062033683
KINETIC STUDIES ON BACILLUS-POLYMYXA NITROGENASE
AUTHOR: HERMANN T E; WILSON P W
JOURNAL: J BACTERIOL 126 (2). 1976 743-750. 1976
FULL JOURNAL NAME: Journal of Bacteriology
CODEN: JOBAA
RECORD TYPE: Abstract

ABSTRACT: Nitrogenase from the facultative anaerobe B. polymyxa was
separated into its component proteins, which were recombined in the ratio
that produced optimal specific activity (125-175 nmol of C2H2 reduced/min
per mg of total protein). The apparent Km's for the Mg-ATP complex,
reducible substrates azide , acetylene and N2, and the nonphysiological
electron donor S2O42- were 0.7, 0.7, 0.2, 0.06 and 0.03 mM, respectively.
These apparent Km values are in reasonable agreement with those reported
for the nitrogenases of Azotobacter vinelandii and Klebsiella pneumoniae.
Either a total lack of cooperativity between binding sites or a single
binding site for reducible substrates is indicated by analysis of Hill
plots. Hill plot slopes of approximately 1.7 suggest that multiple
binding sites exist for ATP and S2O42-.
DESCRIPTORS: AZOTOBACTER-VINELANDII
KLEBSIELLA-PNEUMONIAE SPECIFIC ACTIVITY
MICHAELIS CONSTANTS ATP HYDRO SULFITE
BINDING SITES HILL PLOT
CONCEPT CODES:
10010 Comparative Biochemistry, General
10012 Biochemistry-Gases (1970-)
10506 Biophysics-Molecular Properties and Macromolecules
10802 Enzymes-General and Comparative Studies; Coenzymes
10808 Enzymes-Physiological Studies
13002 Metabolism-General Metabolism; Metabolic Pathways
13003 Metabolism-Energy and Respiratory Metabolism
13010 Metabolism-Minerals
13014 Metabolism-Nucleic Acids, Purines and Pyrimidines
31000 Physiology and Biochemistry of Bacteria
04500 Mathematical Biology and Statistical Methods
10050 Biochemical Methods-General
10060 Biochemical Studies-General
10062 Biochemical Studies-Nucleic Acids, Purines and Pyrimidines
10064 Biochemical Studies-Proteins, Peptides and Amino Acids
10069 Biochemical Studies-Minerals
10804 Enzymes-Methods
32000 Microbiological Apparatus, Methods and Media
BIOSYSTEMATIC CODES:
07200 Eubacteriales (1969-78)
BIOSYSTEMATIC CLASSIFICATION (SUPER TAXA):
Microorganisms
Bacteria

6/9/53 (Item 1 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

07122099 Genuine Article#: 126DA Number of References: 20
Title: Presence of Na+-stimulated V-type ATPase in the membrane of a
facultatively anaerobic and halophilic alkaliphile
Author(s): Kaieda N; Wakagi T; Koyama N (REPRINT)
Corporate Source: CHIBA UNIV,FAC SCI, DEPT CHEM, INAGE KU/CHIBA
2638522//JAPAN/ (REPRINT); CHIBA UNIV,FAC SCI, DEPT CHEM, INAGE
KU/CHIBA 2638522//JAPAN/; UNIV TOKYO,DEPT BIOTECHNOL, BUNKYO KU/TOKYO
1130032//JAPAN/
Journal: FEMS MICROBIOLOGY LETTERS, 1998, V167, N1 (OCT 1), P57-61
ISSN: 0378-1097 Publication date: 19981001
Publisher: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS
Language: English Document Type: ARTICLE
Geographic Location: JAPAN
Subfile: CC LIFE--Current Contents, Life Sciences

Journal Subject Category: MICROBIOLOGY
Abstract: It was found that a facultatively anaerobic and halophilic
alkaliphile, M-12 (Amphibacillus sp.), possesses a Na+-stimulated
ATPase in the membrane. The ATPase activity was inhibited by NO3- and
SCN- which are the inhibitors of V-type ATPase, but not by azide and
vanadate, inhibitors of F-type ATPase and P-type ATPase, respectively.
Upon the incubation of the membrane in buffer containing ATP and MgCl2,
several polypeptides were released from the membrane. Among them, two
major polypeptides with apparent molecular masses of 79 and 55 kDa
crossreacted with an antiserum against the catalytic units (subunits A
and B) of V-type ATPase from Enterococcus hirae. The N-terminal amino
acid sequences of the 79 and 55 kDa polypeptides showed high similarity
to those of subunits A and B of V-type ATPase from Enterococcus hirae,
respectively. M-12 is likely to possess a V-type Na+-ATPase. (C) 1998
Federation of European Microbiological Societies. Published by Elsevier
Science B.V. All rights reserved.
Descriptors--Author Keywords: V-ATPase ; alkaliphile ; halophile ;
Na+-dependent
Identifiers--KeyWord Plus(R): ALKALOPHILIC BACILLUS; PARTIAL-PURIFICATION;
CYTOPLASMIC PH; DEPENDENCE; TRANSPORT
Cited References:
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KRULWICH TA, 1986, V89, P113, J MEMBRANE BIOL
LAEMMLI UK, 1970, V227, P680, NATURE
LOWRY OH, 1951, V193, P265, J BIOL CHEM
MORIYAMA Y, 1989, V264, P3577, J BIOL CHEM
OHTA K, 1975, V86, P259, J GEN MICROBIOL
SUGIYAMA S, 1986, V852, P38, BIOCHIM BIOPHYS ACTA
SUGIYAMA S, 1988, V263, P8215, J BIOL CHEM
VEDDER A, 1934, V1, P141, ANTON LEEUW INT J G
WAKABAYASHI K, 1988, V262, P19, ARCH BIOCHEM BIOPHYS

6/9/54 (Item 2 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

06988870 Genuine Article#: 112BW Number of References: 47
Title: Role of catalase in in vitro acetaldehyde formation by human colonic
contents
Author(s): Tillonen J; Kaihovaara P; JousimiesSomer H; Heine R; Salaspuro
M (REPRINT)
Corporate Source: UNIV HELSINKI,ALCOHOL DIS RES UNIT, TUKHOLMANKATU 8
F/FIN-00290 HELSINKI//FINLAND/ (REPRINT); UNIV HELSINKI,CENT HOSP,
ALCOHOL DIS RES UNIT/HELSINKI//FINLAND/; NATL PUBL HLTH INST,ANAEROBE
REFERENCE LAB/HELSINKI//FINLAND/
Journal: ALCOHOLISM-CLINICAL AND EXPERIMENTAL RESEARCH, 1998, V22, N5 (AUG) , P1113-1119
ISSN: 0145-6008 Publication date: 19980800
Publisher: WILLIAMS & WILKINS, 351 WEST CAMDEN ST, BALTIMORE, MD 21201-2436
Language: English Document Type: ARTICLE
Geographic Location: FINLAND
Subfile: CC LIFE--Current Contents, Life Sciences; CC CLIN--Current
Contents, Clinical Medicine;
Journal Subject Category: SUBSTANCE ABUSE
Abstract: Ingested ethanol is transported to the colon via blood
circulation, and intracolonic ethanol levels are equal to those of the
blood ethanol levels. In the large intestine, ethanol is oxidized by
colonic bacteria, and this can lead to extraordinarily high
acetaldehyde levels that might be responsible, in part, for
ethanol-associated carcinogenicity and gastrointestinal symptoms. It is
believed that bacterial acetaldehyde formation is mediated via
microbial alcohol dehydrogenases (ADHs). However, almost all
cytochrome-containing aerobic and facultative anaerobic bacteria
possess catalase activity, and catalase can, in the presence of
hydrogen peroxide (H2O2), use several alcohols (e.g., ethanol) as
substrates and convert them to their corresponding aldehydes. In this
study we demonstrate acetaldehyde production from ethanol in vitro by

colonic contents in a reaction catalyzed by both bacterial ADH and
catalase. The amount of acetaldehyde produced by the human colonic
contents was proportional to the ethanol concentration, the amount of
colonic contents, and the length of incubation time, even in the
absence of added nicotinamide adenine dinucleotide or H2O2. The
catalase inhibitors sodium azide and 3-amino-1,2,4-triazole (3-AT)
markedly reduced the amount of acetaldehyde produced from 22 mM ethanol
in a concentration dependent manner compared with the control samples
(0.1 mM sodium azide to 73% and 10 mM 3-AT to 67% of control). H2O2
generating system [beta-D(+)-glucose + glucose oxidase] and
nicotinamide adenine dinucleotide induced acetaldehyde formation up to
6- and 5-fold, respectively, and together these increased acetaldehyde
formation up to Ii-fold. The mean supernatant catalase activity was
0.53 +/- 0.1 mu mol/min/mg protein after the addition of 10 mM H2O2,
and there was a significant ($p < 0.05$) correlation between catalase
activity and acetaldehyde production after the addition of the hydrogen
peroxide generating system. Our results demonstrate that colonic
contents possess catalase activity, which probably is of bacterial
origin, and indicate that in addition to ADH, part of the acetaldehyde
produced in the large intestine during ethanol metabolism can be
catalase dependent.

Descriptors--Author Keywords: ethanol ; acetaldehyde ; catalase ; alcohol
metabolism ; colonic bacteria
Identifiers--KeyWord Plus(R): ETHANOL OXIDATION; MOLECULAR
CHARACTERIZATION; BACTERIOCOLONIC PATHWAY; ALDEHYDE DEHYDROGENASES;
ALCOHOL DEHYDROGENASES; BACTERIA; METABOLISM; PURIFICATION;
CONSUMPTION; MICROFLORA

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6/9/55 (Item 1 from file: 35)
DIALOG(R)File 35:Dissertation Abs Online

01452442 ORDER NO: AADAA-I9542935
TRANSFORMATION OF CHLORINATED SOLVENTS BY METAL-REDUCING BACTERIA (SHEWANELLA PUTREFACIENS, POLLUTANT

TRANSFORMATION, BIODEGRADATION)
Author: PETROVSKIS, ERIK AIVARS
Degree: PH.D.
Year: 1995
Corporate Source/Institution: THE UNIVERSITY OF MICHIGAN (0127)
Chair: PETER ADRIAENS
Source: VOLUME 56/08-B OF DISSERTATION ABSTRACTS INTERNATIONAL.
PAGE 4219. 149 PAGES
Descriptors: ENVIRONMENTAL SCIENCES ; ENGINEERING, SANITARY AND MUNICIPAL ; BIOLOGY, MICROBIOLOGY
Descriptor Codes: 0768; 0554; 0410

Investigations of pollutant transformations by pure cultures may enhance our understanding of in situ natural attenuation processes in these environments. Shewanella putrefaciens MR-1, an Fe(III) and Mn(IV) reducing facultative anaerobe , has been shown to dechlorinate tetrachloromethane (CT) to chloroform (24%), after growth under nitrate- or Fe(III)-respiring conditions. Mass balance for carbon included 50% incorporation in biomass, 4% formation of nonvolatile products and 7% mineralization. Product distribution was independent of growth conditions. Amendment of MR-1 cell suspensions with lactate, formate or hydrogen increased CT transformation activity, while methanol did not. The rate and extent of CT transformation increased for MR-1 cells grown with electron acceptors having more positive half-reduction potentials (E$\sp{\circ\prime}$). No inhibition of CT transformation was observed in the presence of nitrate, TMAO or fumarate. However, oxygen did inhibit CT transformation. In the presence of Fe(III), reductive dechlorination was enhanced and resulted in the production of dichloromethane, presumably by abiotic mechanisms involving Fe(II).

CT transformation activity was localized to membrane fractions (78-89%). In membrane fractions, CF production (25-30%) was similar to whole cells. In soluble fractions, CF production was approximately stoichiometric to CT transformation.

The effects of respiratory inhibitors on CT transformation activity have been examined. Rotenone, an inhibitor of NADH dehydrogenase, reduced CT transformation activity in MR-1 whole-cell suspensions using lactate or NADH as an electron donor. Quinacrine, an inhibitor of flavins, enhanced CT transformation activity. No significant inhibitory effect on CT transformation was observed in the presence of pCMPS, sodium azide and sodium cyanide or of cytochrome inhibitors HQNO and Antimycin A. These results suggest that transformation of CT may be mediated by a non-heme electron transfer agent.

Respiratory mutants of MR-1 have been screened for CT transformation activity. Rates of CT transformation for MR-1 terminal reductase mutants, including pigmentless isolates that are presumably deficient in heme cytochromes, were equivalent or greater than those for the MR-1 wild-type strain. MR-1 mutants that did not synthesize menaquinones (MK) and, thereby, lost the ability to couple nitrate-, Fe(III)-, or fumarate reduction for growth, also lost 90% of CT transformation activity. When a MK precursor was added to the MK-deficient mutant during cell growth, CT transformation rates returned to MR-1 wild-type levels. These results indicate that MK or a menaquinol oxidase, but not a terminal reductase, may be responsible for CT transformation by MR-1.

6/9/56 (Item 1 from file: 71)
DIALOG(R)File 71:ELSEVIER BIOBASE

00981431 1998228510
Presence of Nasup +-stimulated V-type ATPase in the membrane of a facultatively anaerobic and halophilic alkaliphile
Kaieda N.; Wakagi T.; Koyama N.
ADDRESS: N. Koyama, Department of Chemistry, Faculty of Science, Chiba University, Yayoi, Inage-ku, Chiba 263-8522, Japan
EMAIL: nkayoma@scichem.c.chiba-u.ac.jp
Journal: FEMS Microbiology Letters, 167/1 (57-61), 1998, Netherlands
CODEN: FMLED
ISSN: 0378-1097
PUBLISHER ITEM IDENTIFIER: S0378109798003735
DOCUMENT TYPE: Article
LANGUAGES: English SUMMARY LANGUAGES: English
NO. OF REFERENCES: 20

It was found that a facultatively anaerobic and halophilic alkaliphile, M-12 (Amphibacillus sp.), possesses a Nasup +-stimulated ATPase in the membrane. The ATPase activity was inhibited by NOsup -inf 3 and SCNsup - which are the inhibitors of V-type ATPase, but not by azide

and vanadate,
inhibitors of F-type ATPase and P-type ATPase, respectively. Upon the
incubation of the membrane in buffer containing ATP and MgClinf 2, several
polypeptides were released from the membrane. Among them, two major
polypeptides with apparent molecular masses of 79 and 55 kDa crossreacted
with an antiserum against the catalytic units (subunits A and B) of V-type
ATPase from Enterococcus hirae. The N-terminal amino acid sequences of the
79 and 55 kDa polypeptides showed high similarity to those of subunits A
and B of V-type ATPase from Enterococcus hirae, respectively. M-12 is
likely to possess a V-type Nasup +-ATPase. Copyright (C) 1998 Federation of
European Microbiological Societies.

DESCRIPTORS:
V-ATPase; Alkaliphile; Halophile; Nasup +-dependent

CLASSIFICATION CODE AND DESCRIPTION:
82.12.11.1 - PROTEIN BIOCHEMISTRY / OTHER PROTEINS / Membrane Proteins /
ATPases
82.2.2 - PROTEIN BIOCHEMISTRY / STRUCTURAL STUDIES / Amino Acid Sequences
(Primary Structure)
82.12.7.3 - PROTEIN BIOCHEMISTRY / OTHER PROTEINS / Microbial Proteins /
Bacterial

6/9/57 (Item 2 from file: 71)
DIALOG(R)File 71:ELSEVIER BIOBASE

00961452 1998208343
Role of catalase in in vitro acetaldehyde formation by human colonic
contents
Tillonen J.; Kaihovaara P.; Jousimies-Somer H.; Heine R.; Salaspuro M.
ADDRESS: Dr. M. Salaspuro, Research Unit of Alcohol Diseases, University of
Helsinki, Tukholmankatu 8 F, 00290 Helsinki, Finland
Journal: Alcoholism: Clinical and Experimental Research, 22/5 (1113-1119),
1998, United States
CODEN: ACRSD
ISSN: 0145-6008
DOCUMENT TYPE: Article
LANGUAGES: English SUMMARY LANGUAGES: English
NO. OF REFERENCES: 47

Ingested ethanol is transported to the colon via blood circulation, and intracolonic ethanol levels are equal to those of the blood ethanol levels.
In the large intestine, ethanol is oxidized by colonic bacteria, and this
can lead to extraordinarily high acetaldehyde levels that might be
responsible, in part, for ethanol-associated carcinogenicity and gastrointestinal symptoms. It is believed that bacterial acetaldehyde
formation is mediated via microbial alcohol dehydrogenases (ADHs). However,
almost all cytochrome- containing aerobic and facultative anaerobic
bacteria possess catalase activity, and catalase can, in the presence of
hydrogen peroxide (Hinf 2Oinf 2), use several alcohols (e.g., ethanol) as
substrates and convert them to their corresponding aldehydes. In this study
we demonstrate acetaldehyde production from ethanol in vitro by colonic
contents in a reaction catalyzed by both bacterial ADH and catalase. The
amount of acetaldehyde produced by the human colonic contents was
proportional to the ethanol concentration, the amount of colonic contents,
and the length of incubation time, even in the absence of added
nicotinamide adenine dinucleotide or Hinf 2Oinf 2. The catalase inhibitors
sodium azide and 3-amino-1,2,4-triazole (3-AT) markedly reduced the
amount of acetaldehyde produced from 22 mM ethanol in a concentration
dependent manner compared with the control samples (0.1 mM sodium azide
to 73% and 10 mM 3-AT to 67% of control). Hinf 2Oinf 2 generating system
[beta-D(+)-glucose + glucose oxidase] and nicotinamide adenine dinucleotide
induced acetaldehyde formation up to 6- and 5-fold, respectively, and
together these increased acetaldehyde formation up to 11-fold. The mean
supernatant catalase activity was 0.53 +/- 0.1 mumol/min/mg protein after
the addition of 10 mM Hinf 2Oinf 2, and there was a significant ($p < 0.05$)
correlation between catalase activity and acetaldehyde production after the
addition of the hydrogen peroxide generating system. Our results
demonstrate that colonic contents possess catalase activity, which probably
is of bacterial origin, and indicate that in addition to ADH, part of the
acetaldehyde produced in the large intestine during ethanol metabolism can
be catalase dependent.

DESCRIPTORS:
Ethanol; Acetaldehyde; Catalase; Alcohol Metabolism; Colonic Bacteria

CLASSIFICATION CODE AND DESCRIPTION:
90.5.4.1 - TOXICOLOGY / EXPERIMENTAL TOXICOLOGY (by agent) / Drugs of Abuse / Alcohol
90.6.7 - TOXICOLOGY / CLINICAL AND EXPERIMENTAL TOXICOLOGY (by target organ) / Digestive

6/9/58 (Item 1 from file: 73)
DIALOG(R)File 73:EMBASE

07437162 EMBASE No: 1998331398
Presence of Nasup +-stimulated V-type ATPase in the membrane of a facultatively anaerobic and halophilic alkaliphile
Kaieda N.; Wakagi T.; Koyama N.
N. Koyama, Department of Chemistry, Faculty of Science, Chiba University, Yayoi, Inage-ku, Chiba 263-8522 Japan
AUTHOR EMAIL: nkayoma@scichem.c.chiba-u.ac.jp
FEMS Microbiology Letters (FEMS MICROBIOL. LETT.) (Netherlands) 1998, 167/1 (57-61)
CODEN: FMLED ISSN: 0378-1097
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DOCUMENT TYPE: Journal; Article
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH
NUMBER OF REFERENCES: 20

It was found that a facultatively anaerobic and halophilic alkaliphile, M-12 (Amphibacillus sp.), possesses a Nasup +-stimulated ATPase in the membrane. The ATPase activity was inhibited by NOsup -inf 3 and SCNsup - which are the inhibitors of V-type ATPase, but not by azide and vanadate, inhibitors of F-type ATPase and P-type ATPase, respectively. Upon the incubation of the membrane in buffer containing ATP and MgClinf 2, several polypeptides were released from the membrane. Among them, two major polypeptides with apparent molecular masses of 79 and 55 kDa crossreacted with an antiserum against the catalytic units (subunits A and B) of V-type ATPase from Enterococcus hirae. The N-terminal amino acid sequences of the 79 and 55 kDa polypeptides showed high similarity to those of subunits A and B of V-type ATPase from Enterococcus hirae, respectively. M-12 is likely to possess a V-type Nasup +-ATPase. Copyright (C) 1998 Federation of European Microbiological Societies.

DRUG DESCRIPTORS:
*adenosine triphosphatase--endogenous compound--ec; *membrane enzyme --endogenous compound--ec
nitrate; thiocyanate; azide ; vanadic acid; adenosine triphosphate; magnesium chloride; polypeptide--endogenous compound--ec
MEDICAL DESCRIPTORS:
*halophilic bacterium; * anaerobic bacterium
amino acid sequence; enzyme inhibition; active transport; nonhuman; article ; priority journal
CAS REGISTRY NO.: 37289-25-1, 9000-83-3 (adenosine triphosphatase); 14797-55-8 (nitrate); 302-04-5 (thiocyanate); 12596-60-0, 14343-69-2 (azide); 12260-63-8, 13981-20-9, 37353-31-4 (vanadic acid); 15237-44-2, 56-65-5, 987-65-5 (adenosine triphosphate); 7786-30-3, 7791-18-6 (magnesium chloride
SECTION HEADINGS:
029 Clinical and Experimental Biochemistry
004 Microbiology: Bacteriology, Mycology, Parasitology and Virology

6/9/59 (Item 2 from file: 73)
DIALOG(R)File 73:EMBASE

07403995 EMBASE No: 1998300856
Role of catalase in in vitro acetaldehyde formation by human colonic contents
Tillonen J.; Kaihovaara P.; Jousimies-Somer H.; Heine R.; Salaspuro M.
Dr. M. Salaspuro, Research Unit of Alcohol Diseases, University of Helsinki, Tukholmankatu 8 F, 00290 Helsinki Finland
Alcoholism: Clinical and Experimental Research (ALCOHOL. CLIN. EXP. RES.) (United States) 1998, 22/5 (1113-1119)
CODEN: ACRSD ISSN: 0145-6008
DOCUMENT TYPE: Journal; Article
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH
NUMBER OF REFERENCES: 47

Ingested ethanol is transported to the colon via blood circulation, and intracolonic ethanol levels are equal to those of the blood ethanol levels. In the large intestine, ethanol is oxidized by colonic bacteria, and this can lead to extraordinarily high acetaldehyde levels that might be responsible, in part, for ethanol-associated carcinogenicity and gastrointestinal symptoms. It is believed that bacterial

acetaldehyde
formation is mediated via microbial alcohol dehydrogenases (ADHs). However,
almost all cytochrome- containing aerobic and facultative anaerobic
bacteria possess catalase activity, and catalase can, in the presence of
hydrogen peroxide (Hinf 2Oinf 2), use several alcohols (e.g., ethanol) as
substrates and convert them to their corresponding aldehydes. In this study
we demonstrate acetaldehyde production from ethanol in vitro by colonic
contents in a reaction catalyzed by both bacterial ADH and catalase. The
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proportional to the ethanol concentration, the amount of colonic contents,
and the length of incubation time, even in the absence of added
nicotinamide adenine dinucleotide or Hinf 2Oinf 2. The catalase inhibitors
sodium azide and 3-amino-1,2,4-triazole (3-AT) markedly reduced the
amount of acetaldehyde produced from 22 mM ethanol in a concentration
dependent manner compared with the control samples (0.1 mM sodium azide
to 73% and 10 mM 3-AT to 67% of control). Hinf 2Oinf 2 generating system
[beta-D(+)-glucose + glucose oxidase] and nicotinamide adenine dinucleotide
induced acetaldehyde formation up to 6- and 5-fold, respectively, and
together these increased acetaldehyde formation up to 11-fold. The mean
supernatant catalase activity was 0.53 +/- 0.1 mumol/min/mg protein after
the addition of 10 mM Hinf 2Oinf 2, and there was a significant ($p < 0.05$)
correlation between catalase activity and acetaldehyde production after the
addition of the hydrogen peroxide generating system. Our results
demonstrate that colonic contents possess catalase activity, which probably
is of bacterial origin, and indicate that in addition to ADH, part of the
acetaldehyde produced in the large intestine during ethanol metabolism can
be catalase dependent.

DRUG DESCRIPTORS:
*acetaldehyde; *catalase--endogenous compound--ec
MEDICAL DESCRIPTORS:
*colon mucosa; *enzyme activation
alcohol metabolism; colon flora; bacterial flora; gastrointestinal symptom;
enzyme activity; supernatant; incubation time; anaerobic bacterium; human
; human tissue; article; priority journal
CAS REGISTRY NO.: 75-07-0 (acetaldehyde); 9001-05-2 (catalase)
SECTION HEADINGS:
040 Drug Dependence, Alcohol Abuse and Alcoholism

6/9/60 (Item 1 from file: 98)
DIALOG(R)File 98:General Sci Abs/Full-Text

03795794 H.W. WILSON RECORD NUMBER: BGSI98045794 (THIS IS THE FULLTEXT)
Multiple-drug resistant enterococci: the nature of the problem and an
agenda for the future.
Huycke, Mark M
Sahm, Daniel F; Gilmore, Michael S
Emerging Infectious Diseases (Emerging Infect Dis) v. 4 no2 (Apr./June '98)
p. 239-49
SPECIAL FEATURES: bibl il ISSN: 1080-6040
LANGUAGE: English
COUNTRY OF PUBLICATION: United States
RECORD TYPE: Abstract; Fulltext RECORD STATUS: Corrected or revised
record
WORD COUNT: 6897

ABSTRACT: Enterococci, leading causes of nosocomial bacteremia, surgical
wound infection, and urinary tract infection, are becoming resistant to
many and sometimes all standard therapies. New rapid surveillance methods
are highlighting the importance of examining enterococcal isolates at the
species level. Most enterococcal infections are caused by Enterococcus
faecalis, which are more likely to express traits related to overt virulence but--for the moment--also more likely to retain sensitivity to at
least one effectivé antibiotic. The remaining infections are mostly caused
by E. faecium, a species virtually devoid of known overt pathogenic traits
but more likely to be resistant to even antibiotics of last resort.
Effective control of multiple-drug resistant enterococci will require 1)
better understanding of the interaction between enterococci, the hospital
environment, and humans, 2) prudent antibiotic use, 3) better contact
isolation in hospitals and other patient care environments, and 4) improved
surveillance. Equally important is renewed vigor in the search for
additional drugs, accompanied by the evolution of new therapeutic paradigms
less vulnerable to the cycle of drug introduction and drug resistance.

TEXT:

The past few years have witnessed increasing interest in enterococci. Until recently, these ordinary bowel commensals languished as misclassified streptococci, commonly perceived "with the exception of endocarditis and rare cases of meningitis ... as not ... a major cause of serious infection" (1). In the last decade, however, enterococci have become recognized as leading causes of nosocomial bacteremia, surgical wound infection, and urinary tract infection (2,3). Two types of enterococci cause infections: 1) those originating from patients' native flora, which are unlikely to possess resistance beyond that intrinsic to the genus and are unlikely to be spread from bed to bed, and 2) isolates that possess multiple antibiotic resistance traits and are capable of nosocomial transmission. The therapeutic challenge of multiple-drug resistant (MDR) enterococci--those strains with significant resistance to two or more antibiotics, often including, but not limited to, vancomycin--has brought their role as important nosocomial pathogens into sharper focus.

The accretion and spread of antibiotic resistance determinants among enterococci, to the point where some clinical isolates are resistant to all standard therapies, highlight both the vulnerability of our present armament as well as the looming prospect of a "postantibiotic era" (4).

This review focuses on the magnitude and nature of the problem posed by enterococci in general, and MDR enterococci in particular. For many points, only representative citations are provided.

HABITAT AND MICROBIOLOGY

Enterococci normally inhabit the bowel. They are found in the intestine of nearly all animals, from cockroaches to humans. Enterococci are readily recovered outdoors from vegetation and surface water, probably because of contamination by animal excrement or untreated sewage (5). In humans, typical concentrations of enterococci in stool are up to 108 CFU per gram (6). Although the oral cavity and vaginal tract can become colonized, enterococci are recovered from these sites in fewer than 20[percent] of cases. The predominant species inhabiting the intestine varies. In Europe, the United States, and the Far East, Enterococcus faecalis predominates in some instances and E. faecium in others (6). Ecologic or microbial factors promoting intestinal colonization are obscure. Of 14 or more enterococcal species (7), only E. faecalis and E. faecium commonly colonize and infect humans in detectable numbers. E. faecalis is isolated from approximately 80[percent] of human infections, and E. faecium from most of the rest. Infections to other enterococcal species are rare.

Enterococci are exceedingly hardy. They tolerate a wide variety of growth conditions, including temperatures of 10[degree]C to 45[degree]C, and hypotonic, hypertonic, acidic, or alkaline environments. Sodium azide and concentrated bile salts, which inhibit or kill most microorganisms, are tolerated by enterococci and used as selective agents in agar-based media.

As facultative organisms, enterococci grow under reduced or oxygenated conditions. Enterococci are usually considered strict fermenters because they lack a Kreb's cycle and respiratory chain (8). E. faecalis is an exception since exogenous hemin can be used to produce d, b, and o type cytochromes (9,10). In a survey of 134 enterococci and related streptococci, only E. faecalis and Lactococcus lactis expressed cytochrome-like respiration (11). Cytochromes provide a growth advantage to E. faecalis during aerobic growth (9). E. faecalis cytochromes are only expressed under aerobic conditions in the presence of exogenous hemin (9,10,12) and, therefore, may promote the colonization of inappropriate sites.

Enterococci are intrinsically resistant to many antibiotics. Unlike acquired resistance and virulence traits, which are usually transposon or plasmid encoded, intrinsic resistance is based in chromosomal genes, which typically are nontransferrable. Penicillin, ampicillin, piperacillin, imipenem, and vancomycin are among the few antibiotics that show consistent inhibitory, but not bactericidal, activity against E. faecalis. E. faecium are less susceptible to b-lactam antibiotics than E. faecalis because the penicillin-binding proteins of the former have markedly lower

affinities
for the antibiotics (13). The first reports of strains highly resistant to
penicillin began to appear in the 1980s (14,15).

Enterococci often acquire antibiotic resistance through exchange of
resistance-encoding genes carried on conjugative transposons, pheromone-responsive plasmids, and other broad-host-range plasmids (6). The
past two decades have witnessed the rapid emergence of MDR enterococci.
High-level gentamicin resistance occurred in 1979 (16) and was quickly
followed by numerous reports of nosocomial infection in the 1980s (17).
Simultaneously, sporadic outbreaks of nosocomial E. faecalis and E. faecium
infection appeared with penicillin resistance due to b-lactamase production
(18); however, such isolates remain rare. Finally, MDR enterococci that had
lost susceptibility to vancomycin were reported in Europe (19,20) and the
United States (21).

Among several phenotypes for vancomycin-resistant enterococci, VanA
(resistance to vancomycin and teicoplanin) and VanB (resistance to
vancomycin alone) are most common (22). In the United States, VanA and VanB
account for approximately 60[percent] and 40[percent] of vancomycin-resistant enterococci (VRE) isolates, respectively (23).
Inducible genes encoding these phenotypes alter cell wall synthesis and
render strains resistant to glycopeptides (22).

VanA and VanB types of resistance are primarily found among
enterococci isolated from clinical, veterinary, and food specimens (24),
but not other common intestinal or environmental bacteria. In the
laboratory, however, these genes can be transferred from enterococci to
other bacteria (22). For example, Staphylococcus aureus has been rendered
vancomycin-resistant through apparent transfer of resistance from E.
faecalis on the surface of membrane filters and on the skin of hairless
obese mice (25), which indicates that there is no biologic barrier to the
emergence of vancomycin-resistant S. aureus. Clinical isolates of highly
vancomycin-resistant S. aureus have yet to be identified, although strains
with reduced susceptibility to vancomycin have appeared (26). The mechanism
of resistance for these strains remains undetermined but does not appear to
involve genes associated with VanA or VanB phenotypes.

EPIDEMIOLOGY

Enterococci account for approximately 110,000 urinary tract infections,
25,000 cases of bacteremia, 40,000 wound infections, and 1,100 cases of
endocarditis annually in the United States (2,27,28). Most infections occur
in hospitals. Although several studies have suggested an increase in
nosocomial infection rates for enterococci in recent years, National
Nosocomial Infections Surveillance system data show little change in the
percentage of enterococcal bloodstream (12[percent] vs. 7[percent]),
surgical site (15[percent] vs. 11[percent]), and urinary tract (14[percent]
vs. 14[percent]) infections over the past 2 decades (3,29). Adequate
surveillance data prior to 1980 are not available. Enterococcal infection
deaths have also been difficult to ascertain because severe comorbid
illnesses are common; however, enterococcal sepsis is implicated in
7[percent] to 50[percent] of fatal cases (6). Several case-control and
historical cohort studies show that death risk associated with antibiotic-resistant enterococcal bacteremia is severalfold higher than
death risk associated with susceptible enterococcal bacteremia (30). This
trend will likely increase as MDR isolates become more prevalent.

Colonization and infection with MDR enterococci occur worldwide. Early
reports showed that in the United States, the percentage of nosocomial
infections caused by VRE increased more than 20-fold (from 0.3[percent] to
7.9[percent]) between 1989 and 1993, indicating rapid dissemination. New
database technologies, such as The Surveillance Network (TSN) Database-USA,
now permit the assessment of resistance profiles according to species. TSN
Database electronically collects and compiles data daily from more than 100
U.S. clinical laboratories, identifies potential laboratory testing errors,
and detects emergence of resistance profiles and mechanisms that pose a
public health threat (e.g., vancomycin-resistant staphylococci).

Data collected by the TSN Database between 1995 and September 1, 1997
were analyzed to determine whether the earlier increase in vancomycin
resistance was unique to vancomycin, whether it represented a continuing

trend, and whether speciation is quantifiably important in analyzing this trend. E. faecalis resistance to ampicillin and vancomycin is uncommon (Figure 1); little change in resistance prevalence occurred from 1995 to 1997. In contrast, E. faecium vancomycin and ampicillin resistance increased alarmingly. In 1997, 771 (52[percent]) of 1,482 of E. faecium isolates exhibited vancomycin resistance, and 1,220 (83[percent]) of 1,474 isolates exhibited ampicillin resistance (Figure 1). E. faecium resistance notwithstanding, E. faecalis remained by far the most commonly encountered of the two enterococcal species in TSN Database. E. faecalis to E. faecium total isolates were approximately 4:1 (Figure 1), blood isolates 3:1, and urine isolates 5:1. This observation underscores important differences in the survival strategies and likelihood of therapeutic success, critical factors usually obscured by lumping the organisms together as Enterococcus species or enterococci. Widespread emergence and dissemination of ampicillin and vancomycin resistance in E. faecalis would significantly confound the current therapeutic dilemma. There is little reason to suspect that vancomycin and ampicillin resistances only provide selective advantage for the species faecium and not faecalis. The relative absence of these resistances in E. faecalis may simply reflect a momentary lack of penetrance and equilibration of the traits. Because of these important differences between the two species, meaningful surveillance of enterococcal resistance must include species identification.

Although exact modes of nosocomial transmission for MDR enterococci are difficult to prove, molecular microbiologic and epidemiologic evidence strongly suggest spread between patients, probably on the hands of health-care providers or medical devices, and between hospitals by patients with prolonged intestinal colonization. At least 16 outbreaks of MDR enterococci have been reported since 1989 (31); all but two were due to E. faecium. This disparity, particularly in view of the higher numbers of clinical E. faecalis isolates, may reflect a reporting bias due to the novelty of the combinations of resistance that occur in E. faecium. When isolates from outbreaks of MDR enterococci have been analyzed by genetic fingerprints, more than half involve clonally related isolates (18,32).

Prior treatment with antibiotics is common in nearly all patients colonized or infected with MDR enterococci (33-35). Clindamycin, cephalosporin, aztreonam, ciprofloxacin, aminoglycoside, and metronidazole use is equally or more often associated with colonization or infection with MDR enterococci than vancomycin use. Other risk factors include prolonged hospitalization; high severity of illness score; intraabdominal surgery; renal insufficiency; enteral tube feedings; and exposure to specific hospital units, nurses, or contaminated objects and surfaces within patient-care areas.

INFECTION CONTROL

Controlling the spread of MDR enterococci among inpatients is difficult. We know relatively little about the biology of enterococcal transmission or the specific microbial factors favoring colonization by exogenous enterococcal strains. Nevertheless, VRE infection control policies, which could apply to MDR enterococci, were recently published by the Hospital Infection Control Practices Advisory Committee (36). Control methods include routine screening for vancomycin resistance among clinical isolates, active surveillance for VRE in intensive care units, contact isolation to minimize person-to-person transmission, and vancomycin restriction.

These measures to limit VRE spread, however, have failed on occasion (35). Not all hospitals can or are willing to perform active surveillance. Because more patients are typically colonized with VRE (3[percent] to 47[percent]) than are infected (35,37,38), and because intestinal colonization can be prolonged, passive surveillance by routine cultures allows colonized inpatients to go unidentified and serve as point sources for continued spread of VRE. Even if all colonized inpatients are successfully identified, VRE may be spread by health-care workers through either inadequate hand washing (39) or through contact with items such as bedrails, sinks, faucets, and doorknobs (enterococci can

persist for weeks on environmental surfaces) (40). Decontamination efforts must be rigorous.

The Hospital Infection Control Practices Advisory Committee strongly recommended restricting oral and parenteral vancomycin to control VRE (36). However, limiting use of vancomycin while ignoring widespread use of other broad spectrum antibiotics likely will not lead to maximal control of VRE or of MDR enterococci.

Antibiotics may promote colonization and infection with MDR enterococci by at least two mechanisms. First, many broad spectrum antibiotics have little or no anti-enterococcal activity, and administration commonly leads to overgrowth of susceptible (or resistant) enterococci at sites at risk for infection. Second, most antibiotics substantially reduce the normal resistance of the intestinal tract to colonization by exogenous organisms (41). Colonization resistance results primarily from the "limiting action" of the normal anaerobic flora, and to a lesser extent from an intact mucosa, gastric acid secretion, intestinal motility, and intestinal-associated immunity (41). Antibiotic-induced alterations in the protective flora of the intestine provide large footholds for colonization with exogenous pathogens such as MDR enterococci (41). Antibiotic restriction programs would be more effective if they included prudent prescribing of all antibiotics, not just single agents such as vancomycin. This approach substantially decreased intestinal colonization with VRE in one hospital pharmacy that restricted vancomycin, cefotaxime, and clindamycin (42).

At a minimum, a successful program for control of MDR enterococci requires effective passive and active surveillance to identify colonized and infected patients, absolute adherence to contact isolation by health-care workers, rigorous decontamination of patient-contact areas and judicious use or restriction of vancomycin and other broad spectrum antibiotics.

THERAPEUTIC APPROACHES

Suitable antibiotics often are not available to treat MDR enterococcal infections, e.g., endocarditis or bacteremia, in the presence of neutropenia. Combinations of penicillin with vancomycin, ciprofloxacin with ampicillin, or novobiocin with doxycycline, among others, have been used (43) but can be unpredictable and remain clinically unproven. In one report chloramphenicol successfully treated chloramphenicol-susceptible infections (44), but these findings await confirmation in controlled trials.

Promising new antibiotics for MDR enterococcal infection under investigation include fluoroquinolones, streptogramins, oxazolidinones, semisynthetic glycopeptides, and glycylcyclines. Clinafloxacin, a fluoroquinolone with improved potency against enterococci compared with ciprofloxacin, has excellent activity against VRE, appears bactericidal in vitro, and has been effective in treatment of enterococcal infections in a murine model (45). Although single-step resistance to clinafloxacin could not be detected in vitro, multistep resistance is readily achieved. Should this agent gain approval for treatment of enterococcal infections, selection for resistance may limit its effectiveness.

Quinupristin/dalfopristin (Synercid) is a combination of streptogramins A and B that inhibits protein synthesis and has a narrower spectrum of activity against enterococci than clinafloxacin (46). Many, but not all, E. faecium isolates with VanA and VanB phenotypes are susceptible (47); however, E. faecalis is uniformly resistant, and superinfection has been reported during therapy (48). In addition, quinupristin/dalfopristin is bacteriostatic only, potentially allowing emergence of resistance (49). For these reasons the drug may have only a limited role in treating MDR enterococcal infections. Novel oxazolidinones and glycylcyclines have also shown potent activity against enterococci, including MDR enterococci (50,51), but await further testing.

The substantial drawback of the broad spectrum approach is that the more organisms affected (both protective commensals as well as pathogens), the more opportunities for resistance to evolve. Broad spectrum antibiotics permit empiric therapy in the absence of a specific diagnosis and generate a more substantial return on investment in the short term. However, broad spectrum antibiotics affect not only disease-causing organisms but also commensals present in numbers large enough to generate resistance by

otherwise rare mutations or genetic exchange events. As long as market forces favor development of broad spectrum therapeutics, a cycle of drug introduction followed by emergence of resistance undoubtedly will continue.

TARGETED THERAPEUTICS

In contrast to the historical reliance on broad spectrum antibiotic therapy, the continuing development and introduction of rapid diagnostic techniques (52) may allow a more focused approach to infectious disease therapy. Any of the myriad microbial-host interactions that subvert the host response or damage tissues during an infection represent potential therapeutic targets. However, many key interactions in disease pathogenesis are specific to the organism involved--a characteristic that is both a strength and a weakness. Because of the specificity of these interactions, rapid and accurate diagnosis is required. However, therapeutics aimed only at interaction between host and a specific pathogen should leave the diverse commensal flora essentially unaffected. As a result, the targeted population would be restricted to the relatively small numbers of disease-producing bacteria and would not likely reach the numbers or diversity required to make development of resistance a statistical probability.

The current spectrum of approaches to identify new antiinfective compounds has two extremes: 1) screening vast libraries of compounds to identify substances that by chance inhibit a microbial property and 2) detailed study of interactions between host and parasite to identify critical events leading to host tissue damage or compromise (53).

With a long-term view toward new therapeutic approaches as well as optimal use of existing therapies, we and others have begun examining in detail the interactions between enterococci and host (6). A major obstacle is that enterococci also form part of the commensal or autochthonous flora; as such, they are nearly devoid of traits traditionally associated with overt pathogens and have subtle interactions with the host. Using inocula with as few as 10 organisms, we have developed sensitive biologic systems for examining the host-parasite interactions (54).

Although E. faecium strains are resistant to vancomycin and ampicillin more often than E. faecalis strains, the relative proportion of infections caused by these species has not dramatically changed in recent years (Figure 1). Since both organisms are frequently isolated from the commensal flora, this bias suggests that E. faecalis traits confer a greater degree of intrinsic virulence, for example, cytolysin production, pheromone-responsive plasmid transfer (and accompanying production of aggregation substance), extracellular superoxide production, and a newly identified surface protein tentatively termed Esp (5,56,57) (Figure 2). These properties provide logical points of departure for developing new targeted therapeutic approaches to enterococcal disease; examination of more subtle interactions between E. faecium and host will follow as an understanding of enterococcal biology evolves.

TARGETING THE E. FAECALIS CYTOLYSIN

Cytolysin is disproportionately expressed by E. faecalis strains associated with disease (5,55,56). This cytolysin causes rupture of a variety of target membranes, including bacterial cells, erythrocytes, and other mammalian cells, with activity observed as a hemolytic zone on some types of blood agar. Cytolysin contributes to the toxicity or lethality of infection in several infection models and is associated with a fivefold increased risk of sudden death from nosocomial bacteremia (54,56-59). Cytolysin also contributes to the appearance of enterococci in a murine bacteremia model (Figure 3; 45,60), an observation consistent with the disproportionate representation of cytolytic strains among human blood isolates (56,62).

Beginning with E.W. Todd in 1934 (63) and culminating in a recent study (64), the E. faecalis cytolysin has now been characterized as a unique, extensively modified bacterial toxin (Figure 4). The cytolysin maturation pathway is ideally designed for therapeutic targeting because the two structural subunits are activated by an extracellular protease, an event that is accessible and potentially inhibitable by a novel

therapeutic. Moreover, the activator protease, CylA, belongs to the
subtilisin class of serine proteases (64), whose structure-function
relationships and inhibitor design we are beginning to understand.
Investigations are in progress to design and test inhibitors of extracellular cytolysin activation to determine whether a reduction by
several logs in the levels of circulating enterococci can be attained as
would be predicted by the observed behavior of cytolysin mutants (Figure
3).

An inhibitor of cytolysin activation, accompanied by appropriate rapid
diagnostics, would be of potential value in treating bacteremia caused by
cytolytic strains of E. faecalis without affecting commensal flora.
Development of resistance should be exceedingly improbable because of the
small number of bacteria targeted and because unlike antibiotics, cytolysin
inhibitors would not act directly on the organism itself.

OTHER ENTEROCOCCAL TARGETS

Several laboratories are using information on the E. faecalis genome and
genomes of other pathogens to identify therapeutic targets (66) and
facilitate studies on pathogenesis for the remaining 60[percent] of
noncytolytic enterococcal infections. The genome of an E. faecalis strain
that caused multiple hospital infections (56) was sampled at high frequency
by sequence analysis. Several sequences appeared to have a role in
host-parasite interaction. The gene specifying Esp encodes an apparent
surface protein of unusual repeating structure (67). Although a role for
this protein in enterococcal infection has yet to be determined, its
distribution among clinical and commensal strains is tantalizing: 29 of 30
strains with this gene were recovered from patients with bacteremia or
endocarditis; one of 34 isolates obtained from healthy volunteers possessed
Esp. The core of this large protein (inferred mass of 202 kDa) consists of
a series of 82 amino acid repeats encoded by highly conserved tandem 246
base pair repeats. Lack of divergence in repeat sequences suggests that
recombination occurs at high frequency, perhaps during infection. Moreover,
the number of repeats observed in homologous genes from different E.
faecalis isolates is 3 to 9 (67). This gene is flanked by a sequence
similar to the transposase of IS905. None of 24 clinical or laboratory E.
faecium isolates had this gene (67; V. Shankar, G. Lindahl, and M. Gilmore,
unpub. data).

A second promising lead involves a series of genes encoding products
highly related to enzymes involved in O-antigen synthesis in gram-negative
bacteria (68). Preliminary evidence suggests that in E. faecalis these
genes contribute to cell wall carbohydrate synthesis and that this
carbohydrate relates to persistence in vivo. A knockout in one of these
genes results in a strain with normal in vitro growth, but after subcutaneous injection, the mutant was more readily cleared than the wild
type parental strain (68). One of the genes studied was present in all E.
faecalis strains examined, whereas another occurs only in E. faecalis
strains that share a periodate-susceptible epitope (68). Collectively,
these data indicate that enzymes for synthesis of E. faecalis surface
carbohydrates are important for persistence in vivo and may represent a
useful therapeutic target. Taking a different approach, Arduino et al.
(69,70) identified a protease-resistant, periodate susceptible substance
associated with some strains of E. faecium, but not E. faecalis, which
conferred resistane to phagocytosis in vitro. The relationship between the
putative carbohydrate of E. faecalis under study above and the inhibitory
substance of E. faecium remains to be determined. It may be found that many
enterococci produce such carbohydrates at biologically significant levels
in vivo, but only some strains of E. faecium do so in vitro.

Finally, recent observations indicate that nearly all E. faecalis
strains, and only a few E. faecium strains, generate substantial extracellular superoxide. When E. faecalis isolates from patients with
endocarditis and bacteremia were compared with isolates from healthy
volunteers (71), on average, extracellular superoxide production was
60[percent] higher among blood isolates than commensal strains. These data
raised several questions: Do E. faecalis that produce larger amounts of
extracellular superoxide possess greater metabolic flexibility, facilitating adaptation to nonintestinal infection sites? Does

free radical production lead to host cell damage, allowing release of normally sequestered nutrients (e.g., hemin) that might promote enhanced E. faecalis growth through cytochrome formation? Might antioxidants modulate colonization or invasive infection? Answers to these questions may provide new insights into the transition from intestinal colonization to infection and may suggest new preventive strategies.

OBSTACLES TO FURTHER DEVELOPMENT

Although important insights into enterococcal biology and pathogenesis are being gleaned from a reverse genetic approach, a paucity of information still exists on how enterococci colonize the intestinal tract and cause infection. For example, do E. faecalis or E. faecium colonize the colon through specific interactions with ligands on human epithelial cells or intestinal mucin? Do MDR enterococci possess alternate binding activities that enable them to colonize the intestinal tract at new sites without competing with the indigenous enterococci? Do probiotics have a role in restoring colonization resistance to an intestinal ecology altered by broad spectrum antibiotics?

Is enough being done to combat the emergence of highly resistant nosocomial pathogens? To effectively compete, industry remains highly responsive to market opportunities. Research in the public sector has been slow to respond, and as a result, our understanding of the biology of enterococcal infection is inadequate. Reasons for the modest public sector response include the following. 1) The emergence of resistant enterococci coincided with a reduction of public support for non-AIDS related infectious disease research. 2) The pathogenesis of nosocomial infection deviates from paradigms established for obligate pathogens. 3) The research infrastructure is relatively small because of the low importance traditionally attached to enterococci as etiologic agents of human disease and the deemphasis on antibiotic resistance research in the 1980s.

CONCLUSIONS

Historically, substantial resources have been invested in developing an in-depth understanding of the molecular biology of model organisms. During the 1960s and 1970s, when gram-negative organisms were leading causes of hospital- and community-acquired infections and gram-positive organisms were typically sensitive to existing antibiotics (72), a sizable investment in gram-negative model organisms was appropriate. However, with the emergence of gram-positive organisms as leading causes of both hospital- and community-acquired infection in the 1990s, a reevaluation of public research priorities is warranted.

Since antibiotic use became widespread 50 years ago, bacteria have steadily and routinely developed resistance. Control of the emergence of resistance will depend on new approaches to prudent antibiotic use in hospitals and clinics, based in part on improved surveillance for MDR enterococci and on better systems to encourage staff adherence to contact isolation procedures. Equally important will be development of new drugs with narrower spectra of activity aimed at known and potentially new targets and the evolution of market conditions that favor their use.

Added material

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Figure 1. Epidemiology of enterococcal infection based on 15,203 susceptibility results obtained by The Surveillance Network (TSN) Database-USA, 1995 to Sep 1, 1997. The increase in total numbers between 1995 and 1996 represents additional reporting centers coming on line. Numbers for 1997 represent total collected for the partial year to Sep 1, 1997.

Figure 2. Virulence traits and their association with enterococcal species.

Figure 3. Cytolysin favors the appearance of circulating enterococci. In this experiment, 107 CFU of E. faecalis, either cytolytic FA2-2(pAM714) (60) or noncytolytic FA2-2(pAM771) (64), were intraperitoneally injected (45) into groups of five BalbC mice. Viable bacteria in liver, spleen, and the bloodstream were enumerated 48 hrs following injection, and significance assessed by Student's t-test. (P. Coburn, L.E. Hancock, and M.S. Gilmore, in preparation).

Figure 4. Cytolysin is expressed and processed through a complex maturation pathway (64). The cytolysin precursors, CylLL and CylLS, are ribosomally synthesized. The putative modification protein, CylM, is required for the expression of CylLL and CylLS in an activatable form, and the secreted forms, CylLL and CylLS were recently shown to possess the amino acid lanthionine as the result of posttranslational modification (64). CylLL and CylLS both are secreted by CylB (65), which is accompanied by an initial proteolytic trimming event (64) converting each to CylLL' and CylLS', respectively. Once secreted, CylLL' and CylLS' are both functionally inactive until six amino acids are removed from each amino terminus. This final step in maturation is catalyzed by CylA (64), a subtilisin-type serine protease. Since this final catalytic event is essential, occurs extracellularly, and is catalyzed by a class of enzyme for which a substantial body of structural information exists, it represents an ideal therapeutic target. As shown in Figure 3, inhibition of cytolysin by mutation (or potentially by therapeutic intervention) results in a reduction by several orders of magnitude in the number of circulating organisms.

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DESCRIPTORS:
Bacteria--Multidrug resistance; Enterococcus

6/9/61 (Item 1 from file: 144)
DIALOG(R)File 144:Pascal

13809642 PASCAL No.: 98-0524984
Presence of Na SUP + -stimulated V-type ATPase in the membrane of a
facultatively anaerobic and halophilic alkaliphile
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University of Tokyo, Yayoi, Bunkyo-ku, Tokyo 113-0032, Japan
Journal: FEMS microbiology letters, 1998, 167 (1) 57-61
ISSN: 0378-1097 CODEN: FMLED7 Availability: INIST-17567A;
354000071189210090
No. of Refs.: 20 ref.
Document Type: P (Serial) ; A (Analytic)
Country of Publication: Netherlands

Language: English
It was found that a facultatively anaerobic and halophilic
alkaliphile. M-12 (Amphibacillus sp.), possesses a Na SUP + -stimulated
ATPase in the membrane. The ATPase activity was inhibited by NO SUB 3 SUP -
and SCN SUP + which are the inhibitors of V-type ATPase. but not by azide
and vanadate, inhibitors of F-type ATPase and P-type ATPase, respectively.
Upon the incubation of the membrane in buffer containing ATP and MgCl SUB 2
, several polypeptides were released from the membrane. Among them, two
major polypeptides with apparent molecular masses of 79 and 55 kDa
crossreacted with an antiserum against the catalytic units (subunits A and
B) of V-type ATPase from Enterococcus hirae. The N-terminal amino acid
sequences of the 79 and 55 kDa polypeptides showed high similarity to those
of subunits A and B of V-type ATPase from Enterococcus hirae, respectively.
M-12 is likely to possess a V-type Na SUP + -ATPase.

English Descriptors: Adenosinetriphosphatase; Sodium; Characterization;
Enzymatic activity; Structural analysis; N terminal-Sequence
Broad Descriptors: Hydrolases; Enzyme; Bacteria; Metabolism; Alkalophily;
Halophily; Hydrolases; Enzyme; Bacterie; Metabolisme; Alcalophilie;
Halophilie; Hydrolases; Enzima; Bacteria; Metabolismo; Alcalofilia;
Halofilia

French Descriptors: Adenosinetriphosphatase; Sodium; Caracterisation;
Activite enzymatique; Analyse structurale; Sequence N terminale;
Amphibacillus

Classification Codes: 002A05B08

6/9/62 (Item 2 from file: 144)
DIALOG(R)File 144:Pascal

13764110 PASCAL No.: 98-0476470
Role of catalase in in vitro acetaldehyde formation by human colonic
contents
TILLONEN J; KAIHOVAARA P; JOUSIMIES-SOMER H; HEINE R; SALASPURO M
Research Unit ofAlcohol Diseases, University Central Hospital of Helsinki
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Institute, Helsinki, Finland
Journal: Alcoholism, clinical and experimental research, 1998, 22 (5)
1113-1119
ISSN: 0145-6008 CODEN: ACRSDM Availability: INIST-17114;
354000070041700200
No. of Refs.: 47 ref.
Document Type: P (Serial) ; A (Analytic)
Country of Publication: United States
Language: English
Ingested ethanol is transported to the colon via blood circulation, and
intracolonic ethanol levels are equal to those of the blood ethanol levels.
In the large intestine, ethanol is oxidized by colonic bacteria, and this
can lead to extraordinarily high acetaldehyde levels that might be
responsible, in part, for ethanol-assodated carcinogenicity and
gastrointestinal symptoms. It is believed that bacterial acetaldehyde
formation is mediated via microbial alcohol dehydrogenases (ADHs). However,
almost all cytochrome-containing aerobic and facultative anaerobic
bacteria possess catalase activity, and catalase can, in the presence of
hydrogen peroxide (H SUB 2 O SUB 2), use several alcohols (e.g., ethanol)
as substrates and convert them to their corresponding aldehydes. In this
study we demonstrate acetaldehyde production from ethanol in vitro by
colonic contents in a reaction catalyzed by both bacterial ADH and
catalase. The amount of acetaldehyde produced by the human colonic contents
was proportional to the ethanol concentration, the amount of colonic
contents, and the length of incubation time, even in the absence of added
nicotinamide adenine dinucleotide or H SUB 2 O SUB 2 . The catalase
inhibitors sodium azide and 3-amino-1,2,4-triazole (3-AT) markedly
reduced the amount of acetaldehyde produced from 22 mM ethanol in a
concentration dependent manner compared with the control samples (0.1 mM
sodium azide to 73% and 10 mM 3-AT to 67% of control). H SUB 2 O SUB 2
generating system (beta -D(+)-glucose + glucose oxidase) and nicotinamide
adenine dinucleotide induced acetaldehyde formation up to 6- and 5-fold,
respectively, and together these increased acetaldehyde

formation up to
11-fold. The mean supernatant catalase activity was 0.53 +- 0.1 mu
mol/min/mg protein after the addition of 10 mM H SUB 2 O SUB 2 , and there
was a significant ($p < 0.05$) correlation between catalase activity and
acetaldehyde production after the addition of the hydrogen peroxide
generating system. Our results demonstrate that colonic contents possess
catalase activity, which probably is of bacterial origin, and indicate that
in addition to ADH, part of the acetaldehyde produced in the large
intestine during ethanol metabolism can be catalase dependent

English Descriptors: Ethanol; Acetaldehyde; Metabolite; Metabolism;
Catalase; Aldehyde dehydrogenase (NAD SUP +); Bacteria; Oxidation; Large
intestin; Colon; Digestive system; Human; In vitro
Broad Descriptors: Peroxidases; Oxidoreductases; Enzyme; Peroxidases;
Oxidoreductases; Enzyme; Peroxidases; Oxidoreductases; Enzima

French Descriptors: Ethanol; Acetaldehyde; Metabolite; Metabolisme;
Catalase; Aldehyde dehydrogenase (NAD SUP +); Bacterie; Oxydation; Gros
intestin; Colon; Appareil digestif; Homme; In vitro

Classification Codes: 002B03F

6/9/63 (Item 3 from file: 144)
DIALOG(R)File 144:Pascal

05845412 PASCAL No.: 84-0346860
Temperature-dependent azide sensitivity of growth and ATPase activity
in the facultative thermophile, Bacillus coagulans
JONES M V; SPENCER W N; EDWARDS C
Univ. Liverpool, dep. microbiology, Liverpool L69 3BX, United Kingdom
Journal: Journal of general Microbiology, 1984, 130 (1) 95-101
ISSN: 0022-1287 Availability: CNRS-4410
No. of Refs.: 24 ref.
Document Type: P (Serial) ; A (Analytic)
Country of Publication: United Kingdom
Language: English
L'inhibition de la croissance de Bacillus coagulans par l'aide de sodium
decroit quand la temperature de croissance augmente alors que le contenu en
cytochrome et particulierement en cytochrome augmente. L'activite de
l'ATPase est sensible a l' azide mais l'inhibition varie a la fois avec la
croissance et la temperature

English Descriptors: Bacillus coagulans; Inhibition; Growth; Temperature;
Enzyme; ATPase; Enzymatic activity; Cytochrome; Anaerobiosis ;
Sensitivity resistance; Metabolism; Bacteria
French Descriptors: Bacillus coagulans; Inhibition; Croissance; Temperature
; Enzyme; ATPase; Activite enzymatique; Cytochrome; Anaerobiose ;
Sensibilite resistance; Metabolisme; Bacterie; Sodium Azoture

Classification Codes: 002A05B13

6/9/64 (Item 1 from file: 149)
DIALOG(R)File 149:TGG Health&Wellness DB(SM)

01086285 SUPPLIER NUMBER: 03904127 (THIS IS THE FULL TEXT)
Geomicrobiology of hydrothermal vents.
Jannasch, Holger W.; Mottl, Michael J.
Science, v229, p717(9)
Aug 23,
1985
PUBLICATION FORMAT: Magazine/Journal ISSN: 0036-8075 LANGUAGE: English
RECORD TYPE: Fulltext TARGET AUDIENCE: Academic
WORD COUNT: 5516 LINE COUNT: 00537

TEXT:
Deep-sea hydrothermal vents were discovered in the 1970's after an
extensive search along the Galapagos Rift (1, 2), a part of the globe-encircling system of sea-floor spreading axes. During the past 7
years, more hydrothermal vent fields have been located along the East
Pacific Rise. They fall into two main groups: (i) warm vent fields with
maximum exit temperatures of 5 degrees to 23 degrees C and flow rates of
0.5 to 2 cm sec.sup.-1 and (ii) hot vent fields with maximum exit
temperatures of 270 degrees to 380 degrees C and flow rates of 1 to 2 m
sec.sup.-1. Hot vent fields commonly include warm- and intermediate-temperature vents ([is less than or =]300 degrees C) ("white
smokers") as well as high-temperature vents (350 degrees [plus-or-minus] 2

degrees C) ("black smokers"). A highly efficient microbial utilization of geothermal energy is apparent at these sites--rich animal populations were found to be clustered around these vents in the virtual absence of a photosynthetic food source (3-5).

Microorganisms, mainly bacteria, are efficient geochemical agents. As prokaryotic organisms, they lack a membrane-bound nucleus and thereby the complex genetic apparatus of the higher, eukaryotic organisms. At the same time, bacteria retain a much wider metabolic diversity than is found in plants and animals. Because of the resulting biochemical versatility of natural microbial populations and the smallness, general resistance, and dispersibility of bacterial cells, these organisms are able to exist in more extreme environments than the higher organisms. Therefore, the occurrence of certain microorganisms at deep-sea vents was predictable; however, their ability to make it possible for higher forms of life to thrive with an unusual efficiency on inorganic sources of energy in the absence of light was entirely unexpected.

Chemosynthesis

The most significant microbial process taking place at the deep-sea vents is "bacterial chemosynthesis." The term was coined by Pfeffer in 1897 (6) in obvious contrast to the then well-known photosynthesis. Both processes involve the biosynthesis of organic carbon compounds from CO.sub.2., with the source of energy being eiher chemical oxidations or light, respectively. More specifically, chemoautotrophy refers to the assimilation of CO.sub.2. and is coupled in some bacteria to chemolithotrophy, the ability to use certain reduced inorganic compounds as energy sources.

In the present-day terminology, the relation between photosynthetic and chemosynthetic metabolism is illustrated in the following schematic equations, where the reduced carbon is represented as a carbohydrate, [CH.sub.2.O]:

From an evolutionary point of view, reactions 1 and 2 above are bridged by the blue-green or cyanobacteria. In aerobic chemosynthesis, the possible electron donors used by a large variety of bacteria are listed in Table 1. Some of them are the same as those used in anaerobic chemosynthesis where free oxygen is replaced by NO.sub.3-, elemental sulfur, SO.sub.4.sup.2-, or CO.sub.2 as electron acceptors. The inorganic sources of energy are used for the production of ATP (adenosine 5'-triphosphate), akin to the use of light in phototrophy.

Differences in the average growth rates of chemolithotrophic bacteria under comparable conditions are determined by the amount of energy required for "reverse electron transfer," a metabolic mechanism required for generating the necessary negative redox potential. Some organisms have the ability to use organic compounds simultaneously as electron sources (mixotrophy). Since in autotrophy carbon (CO.sup.2) must be reduced from a higher oxidative state than organic carbon, more energy is required than in heterotrophy. Therefore, obligate chemoautrophic bacteria generally grow more slowly than heterotrophs or require larger amounts of substrate in terms of energy supply.

During recent years a number of new types of anaerobic chemoautotrophic bacteria have been isolated and described. Among them are methanogens, acetogens, and sulfate-reducing bacteria (7). In addition, it has been shown that certain extremely thermophilic methanogens are able to respire elemental sulfur (8, 9). All these metabolic types are potential catalysts of geochemical transformations at deep-sea vents.

All the inorganic energy sources listed in Table 1 have been found in hydrothermal fluids or in waters surrounding the vents except thiosulfate, the occurrence of which has not been specifically studied. Before discussing those types of bacteria that have been isolated and those microbial processes that have been shown to occur, we will outline the hydrothermal origin and the documented occurrence of the critical inorganic species.

Sources of H.sub.2.S and Structure of the Mixing Region

The chemistry of the vent waters indicates that both warm and hot vent fields are fed at depth by a high-temperature end-member solution at about 350 degrees C and that the mixing of this solution with largely unreacted and unheated ocean bottom water in the shallow regions of the crust is responsible for the wide range of exit temperatures (2, 10). Thus,

chemical species that are nonreactive during mixing define mixing lines as a function of temperature for the warm vent waters. These lines pass through ambient seawater and extrapolate to a composition at 350 degrees C similar to that actually measured in the hot vent waters.

Most of the chemical species thought to participate in microbiological reactions do not exhibit such linear mixing behavior. These species may therefore originate either at depth in the high-temperature end-member solution that has been produced by reaction of heated seawater with crustal rocks (11), or they may originate in the shallow subsea-floor region, either directly from bottom sea-water or as a result of various inorganic and organic reactions that occur on mixing (Fig. 1).

The concentrations of relevant species from the best-studied hot vent fields (those on the East Pacific Rise near 21 degrees N) and warm vent fields (those on the Galapagos Rift near 86 degrees W) are shown in Table 2. Also shown are the results of two model calculations, the first for a conservative mixture of the hot vent waters with ocean bottom water and the second for the same mixture after some simplified inorganic reactions have occurred.

The prominence of H.sub.2.S is obvious from Table 2. There are two possible sources for H.sub.2.S in the hot vent waters: it may be leached from crustal basalts, or it may be produced by reduction of SO.sub.4/.sup.2- from seawater coupled with oxidation of Fe.sup.2+ from basalt to Fe.sup.3+. Both mechanisms are important in laboratory experiments at 300 degrees C and above, but they occur only sluggishly or not at all at lower temperatures (12). It is likely that both mechanisms are important in the natural system as well. The concentration of sulfur in typical mid-ocean ridge basalt ([is approxi.]25 mmol/kg as S.sup.2-) is similar to that in seawater ([is approxi.]28 mmol/kg as SO.sub.4.sup.2-), and seawater circulating through the hydrothermal system of a mid-ocean ridge apparently reacts with an amount of fresh rock about equal to its own mass (10, 13, 14). Although the hot vent waters are essentially free of SO.sub.4.sup.2-, circulating seawater can be expected to lose some or all of its load of SO.sub.4.sup.2- as anhydrite (CaSO.sub.4), which precipitates on heating to temperatures as low as 130 degrees C (15). Thus, little seawater SO.sub.4.sup.2- may be delivered to the deeper, hotter parts of the system where it could be reduced to S.sup.2-. Sulfur isotopic analyses of H.sub.2.S from the hot vent waters and of sulfide minerals from the precipitated vent chimneys indicate that H.sub.2.S is derived mainly from the basalts, but that the seawater source also is important (16).

The conservatively calculated H.sub.2.S concentration in Table 2 for a 12.6 degrees C mixture of hot vent water with seawater is in the same range as those in the warm vent waters at the same temperature. H.sub.2.S undoubtedly is not conservative during subsurface mixing, however, as Fe.sup.2+, O.sub.2, and NO.sub.3- are all heavily depleted in the warm vent waters, presumably as a result of reaction with H.sub.2.S. Examination of the relation between vent temperature and the concentrations of species that react on mixing in the shallow subsea-floor provides insight into the structure of the shallow crustal mixing region and the chemical processes that occur there. This mixing region, with its large area of basalt surfaces, which serve as substrate, and its dual source of electron donors from the hot water end-member and electron acceptors from seawater, is a major site of microbial production.

the generalized relation is shown in Fig. 2. For a given vent field, O.sub.2 and NO.sub.3- decrease linearly from their values in ocean bottom water to zero at characteristic temperatures <20 degrees C that vary from one vent field to another (Table 3). H.sub.2.S decreases linearly with decreasing temperature as O.sub.2 and NO.sub.3- increase, generally going to zero at the bottom-water temperature of 2 degrees C. An inflection typically occurs in the H.sub.2.S-temperature relation where O.sub.2 goes to zero, with the slope of the H.sub.2.S temperature curve becoming steeper at higher temperatures. Other species whose concentrations decrease from their seawater values and extrapolate to zero at temperatures [is less than or =]20 degrees to 30 degrees C in the warm vent waters are chromium, uranium, nickel, copper, cadmium, and selenium (10).

Thus distinct zones exist in the shallow subsea-floor

mixing region
that are characterized by particular redox conditions; in some cases the
boundaries between these zones are abrupt and isothermal (Fig. 2). Edmond
et al. (10) have inferred that a shallow subsurface reservoir at 10 degrees
to 32 degrees C is located beneath the warm vent fields and is being tapped
by the vents. The temperature range of this reservoir is defined by the
lowest temperatures at which specific chemical processes occur. The
inferred processes are listed in Table 3. The minimum temperatures at which
sulfide deposition occurs in the subsea-floor reservoir are those at which
nickel, copper, cadmium, and selenium go to zero; at lower temperatures,
these species are apparently unreactive and thus define mixing lines with
ambient seawater.

The NO.sub.3- concentration extrapolates to zero at a temperature
just slightly lower than the sulfide-related elements and slightly above
the highest temperature sampled; thus the reservoir is free of NO.sub.3-
and is anoxic because of the reaction of these species from seawater with
H.sub.2.S and other reduced species from the hot-water end-member. the
O.sub.2 concentration goes to zero at a temperature 1 degrees to 11 degrees
C lower than NO.sub.3-, depending on the vent field (Table 3).

These observations are best explained in terms of two distinct zones
that are shallower than the reservoir itself, in which the residence time
of the mixed waters is short relative to the rate of reduction of NO.sub.3-
or NO.sub.2- and O.sub.2, respectively. The warmer zone probably consists
of the channels that connect the reservoir to the sea floor, in which
O.sub.2 is reduced completely but NO.sub.3- is largely nonreactive. Both
NO.sub.3- and H.sub.2.S coexist in this zone (Fig. 2), which was frequently
sampled directly. The cooler zone probably consists of the throats of the
vents themselves, in which the residence time is so short that all species,
mix conservatively; H.sub.2.S, O.sub.2 and NO.sub.3- coexist in this zone.

Samples from several vents within a single vent field define single
mixing lines for reactive species. This implies that the temperatures that
bound the various zones are uniform across the area of an individual vent
field. Variation in these characteristic temperatures from one field to
another (Table 3) may reflect to some extent the variations in composition
of the hot-water end-member feeding the various fields. Probably, however,
this variation is mainly a function of the shallow crustal channel geometry
and the distribution of permeability and recharge rates of seawater to the
subsea-floor reservoir. The uniformity of the characteristic temperatures
for different vents within a single vent field reinforces the notion of a
subsurface reservoir created by permeability variations in the shallow
subsea-floor.

Because the inferred reservoir is anoxic, like the water in the
surficial upflow channels, aerobic chemosynthetic microorganisms probably
thrive mainly at the margins of these zones, where downwelling oxygenated
seawater mixes with the major bodies of already mixed and reacted
solutions. Electron-donor species from the reservoir would be available at
these sites.

Sources of Other Chemical Species Used in Chemosynthesis

In addition to H.sub.2.S, the subsea-floor reservoir contains H.sub.2
(17), although in much lower concentrations than would be expected from the
high values in the hot-water end-member (Table 2). When seawater was
reacted with basalts in laboratory experiments (18), the resultant
concentration of H.sub.2 was lower than that in the natural 350 degrees C
solutions. It apparently was controlled by the redox state, which was near
the magnetite-hematite boundary at 350 degrees to 375 degrees C. The
H.sub.2-O.sub.2 redox couple approached equilibrium faster than any other
redox couple. Isotopic data on H.sub.2 from the hot vent waters also
suggest a close approach to equilibrium for H.sub.2-H.sub.2.O (12).
Inorganic reaction of H.sub.2 with O.sub.2 from seawater to relatively low
temperatures during mixing could easily account for the relatively low
H.sub.2 concentrations in the warm vent waters, which may then have been
affected by bacterial reactions.

In contrast to H.sub.2, CH.sub.4 and CO are present at much higher
concentrations in the warm vent waters than would be

expected from the concentrations in the hot vent water (Table 2). CH.sub.4 in the hot vents is almost certainly abiogenic, on the basis of its similar concentration in fresh basalts and its relatively heavy isotopic composition (19), although interpretation of the isotopic data has been questioned (17). No isotopic data are available for CH.sub.4 or CO from the warm vents, but the anomalously high concentrations of these two species could well indicate a primarily biological origin, probably in the anoxic subsea-floor reservoir.

As with NO.sub.3-, CH.sub.4 behaves linearly with temperature over the entire interval sampled (17), indicating that, unlike H.sub.2.S and O.sub.2, it is conserved in the inferred channels to the sea-floor. In at least one warm vent field (Rose Garden), CO apparently is produced in the upflow channels, as indicated by its inflection point and slope when plotted against temperature.

The reservoir also contains Fe.sup.2+ and Mn.sup.2+ in substantial concentrations, derived by leaching from basalt at high temperature. Mn.sup.2+ plots linearly against temperature over the entire interval sampled for the warm vents (10), and these lines extrapolate to concentrations similar to those in the 350 degrees C end-member (Table 2). Thus, Mn.sup.2+ is largely nonreactive in the shallow subsea-floor. Fe.sup.2+, by contrast, is nonlinear over the sampled interval in the same sense as H.sub.2.S (Fig. 2); thus it is being removed from solution in the upflow channels as well as in the reservoir, probably by a combination of sulfide and oxide deposition. It is uncertain to what extent Fe.sup.2+ is utilized in microbiological reactions, as it readily participates in inorganic reactions under these conditions.

Other electron donors present in the subsea-floor reservoir do not originate mainly from the hot-water end-member. NH.sub.4+ and NO.sub.2- were at or below detection limits in the 350 degrees C solutions but were readily measurable in the warm vent waters (Table 2). They almost certainly derive from reduction of seawater NO.sub.3- introduced into the reservoir, by reaction mainly with H.sub.2S. Also present at very low concentrations is N.sub.2.O (17). These species together account for less than 20 percent of the introduced NO.sub.3-; most of the rest is presumably reduced to N.sub.2. NH.sub.4+ and NO.sub.- behave linearly versus temperature over the entire interval sampled for some warm vent fields (for example, Clambake); for others, however (NO.sub.2- in Oyster Beds), they display inflection points indicating their display inflection points indicating their consumption in the upflow channels. Thiosulfate has not been sought, but elemental sulfur has been detected in warm vent effluent as well as in the chimneys of black smokers and white smokers. The slopes of plots of H.sub.2.S versus temperature for those warm vent samples that are free of O.sub.2 suggest that sulfur species with intermediate oxidation states are being formed on mixing as well as SO.sub.4.sup.2-, although SO.sub.4.sup.2- is usually dominant. Seawater also contributes SO.sub.4.sup.2- directly to the subsea-floor reservoir.

Among the electron acceptors, CO.sub.2 is paramount. This species is highly enriched in the hot vent water by the leaching of CO.sub.2 from basalt (19, 20). Its concentration in the warm vent waters is about what it should be if the behavior of CO.sub.2 on mixing is conservative (Table 2).

Microbial Populations of Emitted Vent Waters

Without considering their specific catalytic function, one can assess abundance of natural bacterial populations by determining cell concentrations or by measuring growth rates using unspecific tracers. The milky-bluish waters (Fig. 3A) flowing from some of the warm vents (6 degrees to 23 degrees C, 1 to 2 cm sec.sup.-1) contain between 10.sup.5 and 10.sup.9 cells per milliliter (2, 4, 5). Independent of the temperatures measured, the large range of numbers is due to the dilution of vent water at the point of sampling. Visible bacterial aggregates add to this heterogeneity and may represent dislodged pieces of microbial mats (4, 5).

When contamination by ambient water was strictly prevented, we were unable to find significant numbers of microscopically visible bacteria in hot (338 degrees to 350 degrees C) vent water. In contrast, 4.7 X 10.sup.5 cells were counted in vent water at 304 degrees C (21) when the temperature was

determined from magnesium concentrations (22). This finding indicated an unspecified amount of seawater intrusion prior to or during sampling.

Since aerobic chemosynthesis results in higher productivity than anaerobic chemosynthesis, the availability of the electron donor and oxygen under favorable growth conditions will be decisive. From this point of view, bacterial productivity should be highest in the vicinity of warm vents where the slow emission of sources of reduced chemical energy into oxygenated seawater forms slowly moving plumes. In contrast, the forceful emission of hydrothermal fluid from the hot vents results in a quick dispersal and fast dilution of energy sources in the water column, eventually leading to chemical oxidations. The observation of maximum populations of animals in the immediate vicinity of warm vent plumes and heavy bacterial mats near warm leakages at the base of hot vent chimneys supports these assumptions.

Biomas measurements can also be based on determinations of adenosine triphosphate (ATP) or total adenylates (22). Data of Karl et al. (5) demonstrate that the microbial biomass of warm vent plumes, determined as ATP, was two to three times that of the photosynthetic-heterotrophic microbial populations of surface waters at the same site (Galapagos Rift). The ratio of guanosine 5'-triphosphate to ATP, also measured in this study (5), has been interpreted as an indicator of growth rates. It correlated well with the data derived from biomass determinations (5).

The most recent developments in the measurement of growth rates of natural microbial populations are based on the use of tritiated nucleotides (adenine or thymidine) for incorporation into RNA and DNA (23). It is assumed that the assimilation of these marker substrates does not affect growth by stimulating ATP production. In a recent study with samples collected from a hot smoker orifice, higher adenine incorporation rates were found at 90 degrees C than at 21 degrees and 50 degrees C (24).

In addition to their occurence in warm vent water plumes (Fig. 3A), large microbial populations are also found (i) as mats covering almost indiscriminately all surfaces exposed to warm vent plumes (Fig. 3, B and C) and (ii) in symbiotic tissues within certain vent invertebrates (see below). Quantitative data on microbial activities at these two sites have not yet been obtained.

Sulfur-Oxidizing Bacteria and Rates of Chemosynthesis

The predominant chemosynthetically usable chemical energy at the vents appears in the form of sulfur compounds. This predominance is reflected in the ease and success with which sulfur-oxidizing bacteria can be isolated (25). In general, the types of sulfur bacteria found at the deep-sea vents do not differ greatly from those isolated from other H.sub.S-rich environments. There is one exception to this rule: the common occurrence of the genus Thiobacillus appears to be replaced by a prevalence of the genus Thiomicrospira (25).

Pure-culture isolations resulted in a wide range of metabolic types of sulfur bacteria including acidophilic obligate chemoautotrophs, mixotrophs (which simultaneously assimilate inorganic and organic carbon), and facultative chemoautotrophs (25). Since the presence of organic carbon can be expected to be widespread within the vent communities, the facultative chemoautotrophs may well represent the predominant type of sulfur bacteria. The demonstrated excretion of organic carbon by obligate chemoautotrophs indicates the possible occurrence of these bacteria even in the subsurface vent systems (25). The preference for a neutral pH range favors the faculative (polythionate-producing) chemoautotrophs in the well-buffered seawater environment (26). This biochemical versatility of sulfur bacteria, together with the relatively high concentrations of reduced sulfur compounds, appears to be the key to their predominance at the vents and to their role as primary chemosynthetic producers compared to the other types of chemolithoautotrophic bacteria.

As in the measurement of photosynthesis, CO.sub.2 was used as a substrate to determine rates of chemosynthesis. With the aid of the research submersible Alvin, arrays of six 200-ml syringes were filled in situ from a joint inlet (27). They facilitated replica and control samplings and were used for in situ incubation experiments (Fig. 4). At the base of the 21 degrees N black smoker, the in situ rate of

CO.sub.2 incorporation by natural microbial populations in warm water leakages was approximately 10.sup.-6 [mu]M ml.sup.-1 day.sup.-1 (27). When parallel samples were incubated in the ship's laboratory (atmospheric pressure) at 3 degrees C, the rate was virtually the same (indicating a minimal effect of hydrostatic pressure). This result was corroborated by data on the metabolic rates of a pure culture culture isolate (Thiomicrospira, strain L-12) as affected by pressure (24).

In a second shipboard incubation at 23 degrees C, the in situ temperature of the warm-water leakages, the rate of CO.sub.2 incorporation increased one and a half orders of magnitude (27). This behavior indicates the "mesophilic" growth characteristic of the total natural population. A similar response was found in pure cultures. An addition of 1mM thiosulfate as an accessory energy source in all three experiments resulted in subtantial rate increases. This immediate use of reduced sulfur confirmed the predominance of sulfur-oxidizing bacteria in the natural population (27).

Different types of dense bacterial mats have been observed at various vents (28). The genera Thiothrix and Beggiatoa appear to be predominant according to morphological criteria. During preparations for the isolation of these organisms, the capacities of marine Beggiatoa for the fixation of N.sub.2 and for facultative chemolithoautotrophy have been demonstrated (29). Whitish microbial mats and streamers were commonly observed at the base of hot vents. They represent sites of substantial chemosynthetic production and active grazing by a variety of invertebrates.

Thick mats of Beggiatoa-like filaments, partly floating above the bottom, were observed in situ at exploratory dives at the Guaymas Basin vent site (2000 m deep) in the Gulf of California (30). Collected and fixed specimens showed a filament width of up to 100 [mu]m. At this site, hot vents are overlayed by about 200 m of sediments. A substantial input of photosynthetically produced organic matter from the water column to the sediments further distinguishes this site from all others studied so far.

High concentrations of NHsub3 ([is approx.]4 mM) have also been reported (31), suggesting chemosynthesis by nitrification. A major geochemical-biological study of this site is planned for mid-1985.

Microbial CH.sub.4 Oxidation

Next to reduced sulfur, CH,sub.4, may be a substantial source of energy for chemosynthesis at those deep sea vents where it has been reported to be present in considerable quantities. Although quantitatively less abundant than H.sub.2 in the high-temperature vents, CH.sub.4 is more abundant in the warm vents (Table 2). Evidence for its microbial oxidation is, at this time, stronger than that for H.sub.2 oxidation.

Methanotrophic bacteria are included in the disparate group of the methylotrophic microorganisms, which comprise all those metabolic types that metabolize C.sub.1 compounds (32). CH.sub.4 may serve as the source of both energy and carbon (2CH.sub.4 + 2O.sub.2 right arrow 2[CH.Sub.2.O] + 2H.sub.2O), but CO.Sub.2 may be incorporated as well. All methanotrops are strictly aerobic, often microaerophilic (33), Gram-negative rods, cocci, or vibrios and are characterized by typical intracellular membrane structures.

Methane-utilizing bacteria may also co-oxidize the CO that may occur in vent water (17), without gaining energy in the form of cell carbon through enzymes that normally catalyze other processes (34).

Microbial CH.sub.4 oxidation at the vents was first suggested when the typical morphological characteristics were observed in transmission electron micrographs from bacterial mats (Fig. 3C) (28). Up to 20 percent of the cells surveyed in sections of mats collected from various parts of the vents showed the paired vesicular membranes that distinguish methanotropic cells from similar structures found in ammonium oxidizers.

Both CH.sub.4- and methylamine-oxidizing bacteria were successfully isolated from microbial mats, Filtered vent water, clam gill tissue, And Riftia trophosome (see below), and the pure cultures obtained were preliminary grouped as type I methanotrophs (33).

Hydrogen as a Microbial Source of Energy

Many different types of microorganisms oxidize H.sub.2, but only a few are able to use the energy gained for the fixation of CO.sub.2 and can be described as chemolithoautotrophs (Table 1). Within this

group the term "H.Sub.2 bacteria" is used only for aerobic organisms. Formerly grouped in the genus Hydrogenomonas, the aerobic H.sub.2-oxidizing bacteria are spread over many known genera (35). All of them are facultative autotrophs. As such, they possess ecological advantages similar to those for the facultatively autotrophic sulfur-oxidizing bacteria. They combine the properties of heterotrophic growth with the use of the Calvin cycle enzymes. The net equation for autotrophic growth is 6H.sub.2 + 2O.Sub.2 CO.sub.2 right arrow [CH.sub.2O] 5H.Sub.2O.

Little is known about the ecology of aerobic hydrogen bacteria except that their occurrence in nature is as widespread as that of biological H.sub.2-producing processes. As in the case of sulfide oxidizers, the chemosynthetic use of geothermally produced H2 at the vents represents a primary production of organic carbon. No specific study of aerobic hydrogen bacteria at the vents has yet been undertaken. An organism with a strong growth stimulation by H.sub.2 was isolated incidentally from a Riftia trophosome sample (36).

Anaerobic hydrogen-oxidizing bacteria are known as methanogens and acetogens because of their products (Table 1). They are commonly found at anoxic niches where CO.Sub.2 and H.sub.2 are present as the result of fermentation. In hydrothermal fluid both compounds are produced geothermally. The production of CH.sub.4, H.sub.2, and CO was observed experimentally at about 100[deg.]C in certain media inoculated with samples of black smoker water (37).

An extremely thermophilic methanogen of the genus Methanococcus was isolated from the base of the 21[deg.]N black smoker (Fig. 4) (38). This organism showed an optional growth rate of 0.036 hour.sup.-1 (a doubling time of 28 minutes) at 86[deg.]C. These results demonstrate the existence of a potential biological CH.Sub.4 production at the vents. The absence of isotopic evidence in support of this observation is not necessarily conclusive because of microbial patchiness.

Although denitrifying H.sub.2 oxidizers may exist in vent systems wherever the NO.sub.3./.sup.- -containing bottom seawater mixes with rising hydrothermal fluid, the SO.sub.4./.sup.2- - and sulfur-reducing equivalents are geochemically more significant. Both metabolic types of bacteria do exist but have not yet been isolated from vent waters. The respiration of elemental sulfur has recently been demonstrated to be a common property of extremely thermophilic methanogens and other archaebacteria (8, 9). Above temperatures of [is approx.]80[deg.]C, this microbial sulfur respiration occurs in addition to an abiological reduction.

Microbial Iron and Manganese Oxidation

Deposits of iron and manganese oxides cover most surfaces exposed intermittently to plumes of hydrothermal and bottom seawater or to mixes of the two. The color of these encrustations ranges from almost black to light brown. Scanning electron microscopy reveals dense microbial mats. A large variety of microbial forms are deeply embedded in the metal oxide deposits (Fig. 3, B and C).

Not enough data exist to permit estimates of the rate of mat formation. However, when various types of materials (glass, plexiglass, steel, membrane filters, and clam shells) enclosed in a protective rack were placed into the opening of an active warm (21[deg.]C) vent for [is approx.]10 months, all surfaces were evenly blackened (28, 30).

Nondispersive x-ray spectroscopy showed a decrease of the Fe.sup.2+./.Mn.sup.2+ ratio in these layers with increasing distance from vent openings (28), And observation attributable to the different solubility products of the two metals. X-ray diffraction determinations of the deposits resulted in a correlation with the mineral todorokite, (Mn, Fe, Mg, Ca, K, Na.sub.2) . (Mn.sub.5O.sub.12) . 3H.Sub.2O, which, in its fine-grained and poorly crystalline state, is characteristic of marine ferromanganese deposits.

The role of bacteria in the oxidative deposition of iron is difficult to prove in neutral or alkaline waters where Fe.sup.2+ undergoes rapid spontaneous oxidation in contact with dissolved oxygen. Heterotrophically growth bacteria have been shown to accumulate Fe.sup.3+ deposits, but no physiological significance of this process has ever been demonstrated in the marine environment.

Although iron lithotrophy has been demonstrated for acid freshwaters

and soils true manganese lithotrophy has not been proven (39). The
oxidation of Mn.sup.2+ in seawater (pH[is approx.]8.1) is more likely than
the biological oxidation of Fe.sup.2+. Two bacterial isolates from the
Galapagos Rift vent region oxidized Mn.sup.2+ wither in growing cultures or
in cell extracts (39). The oxidation was heat-labile and inhibited by
azide (NaN3), potassium cyanide (KCNe, and antimycin A. The "oxydase" was
inducible by reduced manganese and was not constitutive as in isolates
obtained from manganese nodules. Since ATP synthesis was coupled with
Mn.sup.2+ oxidation it appears that Mn.sup.2+ - oxidizing bacteria to
contribute to the chemosynthetic production at deep-sea hydrothermal vents.

The Role of Elevated Temperatures

The transfer of thermal to chemical energy takes place at temperatures above 350[deg.]C (Fig. 1). Thermophilic CO.sub.2-,
SO.Sub.4./.sup.2- -, and S.sup.0 -reducing bacteria that use H.sub.2 as the
source of electrons (Table 1) are the best candidates for possible
microbial activities in hot zones where bottom seawater mixes below the
surface with rising hydrothermal fluid. Microbial growth has been measured
so far at temperatures up to 110[deg.]C in cultures of extremely
thermophilic bacteria isolated from shallow and deep marine hot vents (40).

The free O.Sub.2 in this mix of hydrothermal fluid and bottom
seawater may be quickly consumed biologically as well as chemically, and
both aerobic and anaerobic microorganisms may exist in subsurface vent
systems. Most aerobic bacterial isolates obtained from the turbid water
emitted by some of the Galapagos Rift warm vents were "mesophilic," that
is, exhibited growth optima at temperatures of 25[deg.] to 35[deg.]C (24).
"Extremely thermophilic" isolates obtained from the various types of
shallow and deep hot vents are all anaerobic with growth ranges from
65[deg.] to 110[deg.] and growth optima from 86[deg.] to 105[deg.]C (40).
Most of these isolates belong to the "archaebacteria," which are
distinguished from the "eubacteria" and from all eukaryotic organisms by
their specific ribosomal RNA nucleotide sequences (41).

A heterotrophic bacterium that grows on a complex organic medium
(peptone and yeast extract) in a temperature range from 55[deg.] to
98[deg.]C with an optimum at [is approx.]88[deg.]C has recently been
isolated from a shallow marine hot spring as well as from deep-sea vents
(40. It has the facultative respiration of elemental sulfur and some
other characteristics in common with the methanogenic archaebacteria (19).
The methanogenic vent isolate discussed above (38) differs from all other
archaebacteria in having a unique macrocyclic glycerol diether instead of a
tetraether as the polar membrane lipid (42), which is suspected of
affecting the membrane fluidity at high temperatures.

Bacterial growth at temperatures up to 250[deg.] by a natural
population collected from a hot vent has also been reported, but the
experimental proof of this study is still being contested (21). Other
studies with natural populations collected from the immediate vicinity of
hot ents resulted in the microbial production of gases at 100[deg.]C (37)
and in the incorporation of adenine into RNA and DNA at rates that were
higher at 90[deg.]C than at 21[deg.] and 50[deg.] (24). It has also been
spectulated that the particular conditions of deep-sea hydrothermal vents
might lead to a synthesis of organic compounds and ultimately to the origin
of life (43).

Thorough analysis of particulate organic carbon has only been done at
considerable distances from warm vent emissions (44). The results
demonstrated a rather quick passage and complete transformation of
microbially produced organic compounds into those characteristic of certain
grazers (zooplankton). Concern about bacterial growth at hot vents is not
so much a question of whethr there is a substantial addition to primary
production but rather the question of the problem of biological activity at
an upper temperature limit per se.

In the early spring of 1984, dense communities of marine invertebrates were also discovered at a depth of 3200 m at the base of the
West Florida Escarpment, a site without volcanic or geothermic activity
(45). In this area H.sub.2S-containing ground water with a salinity about
one-third higher than that of the ambient seawater seeps from jointed
limestone formations. The types of animals found here are

similar to those
described from the vent sites of the East Pacific Rise, but the individuals
as well as the total quantities are smaller. The presense of H.sub.S has
not been measured, but it is inferred from the odor of the collected
samples. The temperatures of these nongeothermal seepages are near ambient,
that is, about 0.15[deg.]C above ambient when measured at a depth of 10 cm
in the sediment.

From the distributing pattern of invertebrates at the tectonic vent
sites, it appears that the spotty occurrence of elevated temperature is of
secondary importance for the abundance of these populations. The overriding
factors seem to be the availability of inorganic chemical species and the
efficiency of their use in chemosynthesis.

Symbiotic Chemosynthesis

One major evolutionary development is responsible for the unusual
amounts of biomass found at the deep-sea vents: a new type of symbiosis is
not commonly a topic of geomicrobiology, but this newly discovered highly
efficient transformation of geothermal or geochemical energy for the
production of organic carbon poses a new situation.

The predominant part of the biomass observed at the warm deep-sea
vents is generated by the symbiotic association of prokarvotic cells in the
clam Calpytogena magnifica and the pogonophoran tube worm Riftia pachyptila
(46) (Fig. 4). The microbial symbionts have not yet been isolated, but
their prokaryotic nature, DNA base ratio, genome size, and enzymatic
activities identify them as bacteria (36, 47). They are found within the
gill cells of C. magnifica and, as a separate "trophosome" tissue, within
the body cavity of R. pachyptila. The trophosome may amount to 60 percent
of the worm's wet weight.

The animal's dependence on the microbial symbiont has developed to
the point where all ingestive and digestive morphological features have
been lost. Through an active blood system the animal provides the bacteria
in the trophosome with H.sub.2S and free O.sub.2. It appears that the
spontaneous reaction of the two dissolved gases is prevented or slowed by
the presence of an HS.sup.- -binding protein (48). The isolation of
CH.sub.4.-oxidizing bacteria from Calyptogena gill tissue and Riftia
trophosome (33) indicates, but certainly not conclusively, that
chemosynthesis by CH.sub.4 assimilation (ribulose monophosphate pathway)
may also take place. Enzymes associated with both the ATP-producing system
and the Calvin cycle have been found in Riftia and Calyptogena.
Physiological work on purified preparations of symbionts from Riftia and
the newly described vent mussel Bathymodiolus thermophilus (49) showed that
their chemoautotrophic activities differ greatly with respect to
temperature and the type of electron donor used (50).

Probably because of heavy predation of dying vent communities,
fossilized animal remains in metal-rich deposits of ancient sea-floor
spreading centers and presently mined ophiolites have only rarely been
found (51). Evidence for microbial activities at similar sites has been
based on the results of sulfur isotope analyses (52).

The most significant geomicrobiological point of the deep-sea vent
discovery is the dependence of entire ecosystems on geothermal
(terrestrial) rather than solar energy. Were a catastrophic darkening of
the earth's surface to occur (53), the chance of survival of such
ecosystems is the highest of any community in the biosphere. The
chemosynthetic existence of organisms in the deep sea also suggests a
possible occurence of similar life forms in other planetary settings where
water may be present only in the absence of light. It is surprising that,
as far as we know, science fiction writers did not turn their attention to
geochemically supported complex forms of life until such forms were
actually discovered in the deep sea.

CAPTIONS: Electron sources and types of chemolithotropic bacteria
potentially occurring at hydrothermal vents. (table); Schematic diagram
showing inorganic chemical processes occurring at warm- and hot-water vent
sites. (chart); Comparison of the compositions of actual warm vent water at
several vent fields . (table); Tenperatures at which the concentration of
various species in seawater decrease to zero in warm vent fields on the
Galapagos Rift near 86 degrees W. (table); Relation between temperature and
the concentrations of oxygen, nitrous oxide, abd hydrogen sulfide defined
by samples from individual vents in a single warm vent field.

(graph)

SPECIAL FEATURES: illustration; table; chart; photograph; graph
DESCRIPTORS: Microbiological research; Hydrothermal deposits--Research;
Galapagos Rift--Environmental aspects
FILE SEGMENT: MI File 47

6/9/65 (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10006766 99001631 PMID: 9785452
Presence of Na(+)-stimulated V-type ATPase in the membrane of a
facultatively anaerobic and halophilic alkaliphile.
Kaieda N; Wakagi T; Koyama N
Department of Chemistry, Faculty of Science, Chiba University, Japan.
FEMS microbiology letters (NETHERLANDS) Oct 1 1998, 167 (1) p57-61,
ISSN 0378-1097 Journal Code: 7705721
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS
It was found that a facultatively anaerobic and halophilic
alkaliphile, M-12 (Amphibacillus sp.), possesses a Na(+)-stimulated ATPase
in the membrane. The ATPase activity was inhibited by NO3- and SCN- which
are the inhibitors of V-type ATPase, but not by azide and vanadate,
inhibitors of F-type ATPase and P-type ATPase, respectively. Upon the
incubation of the membrane in buffer containing ATP and MgCl2, several
polypeptides were released from the membrane. Among them, two major
polypeptides with apparent molecular masses of 79 and 55 kDa crossreacted
with an antiserum against the catalytic units (subunits A and B) of V-type
ATPase from Enterococcus hirae. The N-terminal amino acid sequences of the
79 and 55 kDa polypeptides showed high similarity to those of subunits A
and B of V-type ATPase from Enterococcus hirae, respectively. M-12 is
likely to possess a V-type Na(+)-ATPase.
Tags: Comparative Study
Descriptors: *Gram-Positive Endospore-Forming Rods--enzymology--EN;
*Proton-Translocating ATPases--metabolism--ME; Amiloride--pharmacology--PD;
Amino Acid Sequence; Catalytic Domain; Enterococcus--enzymology--EN;
Enterococcus--genetics--GE; Enzyme Inhibitors--pharmacology--PD; Gram-Positive Endospore-Forming Rods--genetics--GE; Immunochemistry; Molecular
Sequence Data; Molecular Weight; Nitrates--pharmacology--PD;
Proton-Translocating ATPases--antagonists and inhibitors--AI;
Proton-Translocating ATPases--genetics--GE; Sequence Homology, Amino Acid;
Sodium--pharmacology--PD; Species Specificity; Thiocyanates--pharmacology
--PD
CAS Registry No.: 0 (Enzyme Inhibitors); 0 (Nitrates); 0
(Thiocyanates); 2609-46-3 (Amiloride); 7440-23-5 (Sodium)
Enzyme No.: EC 3.6.1.- (vacuolar H+-ATPase); EC 3.6.3.14
(Proton-Translocating ATPases)
Record Date Created: 19981120

6/9/66 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09969063 98392884 PMID: 9726283
Role of catalase in in vitro acetaldehyde formation by human colonic
contents.
Tillonen J; Kaihovaara P; Jousimies-Somer H; Heine R; Salaspuro M
Research Unit of Alcohol Diseases, University Central Hospital of
Helsinki, Finland.
Alcoholism, clinical and experimental research (UNITED STATES) Aug 1998
, 22 (5) p1113-9, ISSN 0145-6008 Journal Code: 7707242
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS
Ingested ethanol is transported to the colon via blood circulation, and
intracolonic ethanol levels are equal to those of the blood ethanol levels.
In the large intestine, ethanol is oxidized by colonic bacteria, and this
can lead to extraordinarily high acetaldehyde levels that might be
responsible, in part, for ethanol-associated carcinogenicity and
gastrointestinal symptoms. It is believed that bacterial acetaldehyde
formation is mediated via microbial alcohol dehydrogenases (ADHs). However,
almost all cytochrome-containing aerobic and facultative

anaerobic
bacteria possess catalase activity, and catalase can, in the presence of
hydrogen peroxide (H2O2), use several alcohols (e.g., ethanol) as
substrates and convert them to their corresponding aldehydes. In this study
we demonstrate acetaldehyde production from ethanol in vitro by colonic
contents in a reaction catalyzed by both bacterial ADH and catalase. The
amount of acetaldehyde produced by the human colonic contents was
proportional to the ethanol concentration, the amount of colonic contents,
and the length of incubation time, even in the absence of added
nicotinamide adenine dinucleotide or H2O2. The catalase inhibitors sodium
azide and 3-amino-1,2,4-triazole (3-AT) markedly reduced the amount of
acetaldehyde produced from 22 mM ethanol in a concentration dependent
manner compared with the control samples (0.1 mM sodium azide to 73% and
10 mM 3-AT to 67% of control). H2O2 generating system [beta-D(+)-glucose +
glucose oxidase] and nicotinamide adenine dinucleotide induced acetaldehyde
formation up to 6- and 5-fold, respectively, and together these increased
acetaldehyde formation up to 11-fold. The mean supernatant catalase
activity was 0.53+/-0.1 micromol/min/mg protein after the addition of 10 mM
H2O2, and there was a significant ($p < 0.05$) correlation between catalase
activity and acetaldehyde production after the addition of the hydrogen
peroxide generating system. Our results demonstrate that colonic contents
possess catalase activity, which probably is of bacterial origin, and
indicate that in addition to ADH, part of the acetaldehyde produced in the
large intestine during ethanol metabolism can be catalase dependent.
Tags: Female; Human; Male; Support, Non-U.S. Gov't
Descriptors: *Acetaldehyde--pharmacokinetics--PK;
*Bacteria--enzymology
--EN; *Catalase--physiology--PH; *Colon--microbiology--MI;
*Digestive Tract
Contents--microbiology--MI;
*Ethanol--pharmacokinetics--PK; Adult; Aged;
Alcohol Dehydrogenase--physiology--PH; Hydrogen
Peroxide--metabolism--ME;
Middle Age
CAS Registry No.: 64-17-5 (Ethanol); 75-07-0
(Acetaldehyde);
7722-84-1 (Hydrogen Peroxide)
Enzyme No.: EC 1.1.1.1 (Alcohol Dehydrogenase); EC
1.11.1.6 (Catalase)
Record Date Created: 19981215

6/9/67 (Item 3 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07861147 93393353 PMID: 1285025
Efficiency of inhibitors (phenylethanol, nalidixic acid, sodium azide
)in the isolation of strictly anaerobic bacteria from a polymicrobial
specimen]
Efficacite des inhibiteurs (P.E.A, Ac Nal, Az de Na) dans l'isolement des
bacteries anaerobies strictes, a partir d'un prelevement polymicrobien.
Merad A S; Ghemati M
Laboratoire des Anaerobies, Institut Pasteur d'Algerie, Alger.
Archives. Institut Pasteur d'Algerie (ALGERIA) 1992, 58 p161-8,
ISSN 0020-2460 Journal Code: 0373031
Document type: Journal Article ; English Abstract
Languages: FRENCH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS
The efficacy of inhibitors (PEA, Ac Nal, Az de Na) in the isolation of
strict anaerobes in polymicrobial sample. The efficacy of three
inhibitors to select strict anaerobic bacteria in the polymicrobial
sample had been studied. First step: The most frequent anaerobes
encountered in the infections are isolated in the agar Columbia containing
the different inhibitors. This step allowed us to check the inhibition of
the germ we have to isolate. Next step: polymicrobial mixtures were made.
The composition of which is very similar to the samples we receive in the
laboratory. The swarming Proteus is the facultative anaerobic germ
which gives us difficulties when isolating strict anaerobic bacteria.
Then, the different mixtures were isolated separately in the agar in which
the inhibitors were added. The plates containing Azide of Na and PEA gave
us the best results.
Tags: Comparative Study
Descriptors: Azides --pharmacology--PD; *Bacteria, Anaerobic
--isolation and purification--IP; *Bacteriological Techniques;
*Nalidixic
Acid--pharmacology--PD; *Phenylethyl
Alcohol--pharmacology--PD; Bacteria,
Anaerobic --drug effects--DE; Culture Media; Sodium
Azide ; Species

Specificity
CAS Registry No.: 0 (Azides); 0 (Culture Media); 26628-22-8 (Sodium Azide); 389-08-2 (Nalidixic Acid); 60-12-8 (Phenylethyl Alcohol)
Record Date Created: 19931021

6/9/68 (Item 4 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06741269 91054124 PMID: 2241708
Characterization of a gram-positive bacterium from the proventriculus of budgerigars (Melopsittacus undulatus).
Scanlan C M; Graham D L
Department of Veterinary Microbiology and Parasitology, Texas A&M University, College Station 77843-4467.
Avian diseases (UNITED STATES) Jul-Sep 1990, 34 (3) p779-86, ISSN 0005-2086 Journal Code: 0370617
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS
The cellular, cultural, and biochemical characteristics of eight isolates of a large gram-positive bacillus that are commonly observed as apparently normal flora in the proventriculus of budgerigars (Melopsittacus undulatus) were determined. The bacterium was highly pleomorphic and changed markedly in both diameter and length when subcultured on agar media. The bacterium was facultative anaerobic and capnophilic, hemolytic on blood agar, and formed flat colonies with irregular edges after incubation for several days. All isolates grew on sodium azide agar but did not grow on MacConkey agar. The isolates were catalase-negative and oxidase-negative and did not reduce nitrate. All isolates failed to utilize arginine, lysine, ornithine or tryptophane but produced acid from glucose, galactose, levulose, maltose, melibiose, starch, and sucrose. All isolates produced acetoin from glucose and hydrolyzed esculin. The eight isolates could not be identified to either genus or species level based on the descriptions of currently classified organisms in the division Firmicutes as described in Bergey's Manual of Systematic Bacteriology.
Tags: Animal
Descriptors: *Gram-Positive Bacteria--physiology--PH; *Proventriculus --microbiology--MI; *Psittacines--microbiology--MI; Anti-Infective Agents --pharmacology--PD; Gram-Positive Bacteria--drug effects--DE; Gram-Positive Bacteria--growth and development--GD
CAS Registry No.: 0 (Anti-Infective Agents)
Record Date Created: 19901207

6/9/69 (Item 5 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

02326397 76167486 PMID: 770451
Kinetic studies of Bacillus polymyxa nitrogenase.
Hermann T E; Wilson P W
Journal of bacteriology (UNITED STATES) May 1976, 126 (2) p743-50, ISSN 0021-9193 Journal Code: 2985120R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS
Nitrogenase from the facultative anaerobe Bacillus polymxa was separated into its component proteins, which were recombined in the ratio that produced optimal specific activity (125 to 175 nmol of C2H2 reduced/min per mg of total protein). The apparent Michaelis constants (Km)for the magnesium adenosine triphosphate complex, reducible substrates azide , acetylene, and N2 and the nonphysiological electron donor hydrosulfite (S2O42-) were determined to be 0.7, 0.7, 0.2, 0.06, and 0.03 MM, respectively. These apparent Km values are in reasonable agreement with those reported for the nitrogenases of Azotobacter vinelandii and Klebsiella pneumoniae. Either a total lack of cooperativity between binding sites or a single binding site for reducible substrates is indicated by analysis of Hill plots. Hill plot slopes of approximately 1.7 suggest that multiple binding sites exist for both ATP and S2O42-.
Tags: Support, U.S. Gov't, Non-P.H.S.
Descriptors: *Bacillus--enzymology--EN; *Nitrogenase--metabolism--ME; Acetylene--metabolism--ME; Adenosine Triphosphate--metabolism--ME; Anaerobiosis ; Azides --metabolism--ME; Azotobacter--enzymology--EN; Binding Sites; Cell-Free System; Kinetics; Klebsiella pneumoniae --enzymology--EN; Nitrogen--metabolism--ME; Species Specificity; Sulfites --metabolism--ME
CAS Registry No.: 0 (Azides); 0 (Sulfites); 56-65-5

(Adenosine
Triphosphate); 74-86-2 (Acetylene); 7727-37-9 (Nitrogen)
Enzyme No.: EC 1.18.6.1 (Nitrogenase)
Record Date Created: 19760706

6/9/30 (Item 30 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

11646647 BIOSIS NO.: 199800428378
Endogenous SecA catalyzes preprotein translocation at SecYEG.
AUTHOR: Eichler Jerry; Rinard Kate; Wickner William(a)
AUTHOR ADDRESS: (a)Dep. Biochemistry, Dartmouth Med. Sch., 7200 Vail,
Hanover, NH 03755-3844**USA
JOURNAL: Journal of Biological Chemistry 273 (34):p21675-21681 Aug. 21, 1998
ISSN: 0021-9258
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: SecA is found in the cytosol and bound to the plasma membrane of
Escherichia coli. Binding occurs either with high affinity at SecYEG or
with low affinity to lipid. Domains of 65 and 30 kDa of SecYEG-bound SecA
insert into the membrane upon interaction with preprotein and ATP. Azide
blocks preprotein translocation, in vivo and in vitro, through
interacting with SecA and preventing SecA deinsertion. This provides a
measure of the translocation relevance of each form of SecA membrane
association. We now report that azide acts exclusively on SecA that is
cycling at SecYEG and has no effect on SecA lipid associations. SecA
molecules recovered with sucrose gradient-purified inner membrane
vesicles ("endogenous" SecA) support translocation at the same rate as
"added" SecA molecules bound at SecYEG. Both endogenous and added SecA
yield the same proteolytic fragments, which are distinct from those
obtained from SecA once it has inserted into membranes at SecYEG or from
SecA at lipidic sites. Endogenous and added SecA differ, however, in
their resistance to urea extraction. The translocation supported by
either endogenous or added SecA is blocked by azide or by antibody to
SecY. We conclude that SeeA functions in preprotein translocation only
through cycling at SecYEG.

REGISTRY NUMBERS: 14343-69-2: AZIDE
DESCRIPTORS:
MAJOR CONCEPTS: Biochemistry and Molecular Biophysics; Membranes (Cell
Biology)
BIOSYSTEMATIC NAMES: Enterobacteriaceae--Facultatively Anaerobic
Gram-Negative Rods, Eubacteria, Bacteria, Microorganisms
ORGANISMS: Escherichia-coli (Enterobacteriaceae)
ORGANISMS: PARTS ETC: plasma membrane
BIOSYSTEMATIC CLASSIFICATION (SUPER TAXA): Bacteria; Eubacteria;
Microorganisms
CHEMICALS & BIOCHEMICALS: azide ; SecA; SecYEG
MISCELLANEOUS TERMS: preprotein translocation
CONCEPT CODES:
10060 Biochemical Studies-General
30000 Bacteriology, General and Systematic
BIOSYSTEMATIC CODES:
06702 Enterobacteriaceae (1992-)

6/9/11 (Item 11 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

11981903 BIOSIS NO.: 199900262422
Superoxide dismutase and catalase in marine bioluminescent bacteria.
AUTHOR: Gonzalez-Lama Z(a); Diez del Pino A(a)
AUTHOR ADDRESS: (a)Microbiologia, Departamento de Ciencias Clinicas,
Facultad de Ciencias de la Salud, Universidad **Spain
JOURNAL: Boletin Instituto Espanol de Oceanografia 12 (2):p131-137 1996
ISSN: 0074-0195
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: Spanish; Non-English
SUMMARY LANGUAGE: English; Spanish

ABSTRACT: Catalase and superoxide dismutase (SOD) were studied in strains
of marine bioluminescent bacteria. We found several isozymes of catalase
in these strains and only one isozyme of superoxide dismutase. We
observed that catalase levels rose as bioluminiscence emission fell. A
dark strain of Photobacterium phosphoreum var. K showed the maximun
levels of catalase. There are two types of catalases in this strain: an
isozyme of pI 7.2 inhibited by 3-amino, 1, 2, 4-triazole and others
isozymes resistent to this inhibitor. All isozymes of catalase from these
bioluminescent marine bacteria are hemo-proteins, since they were

inhibited by cianyde and azide . The single isozyme of SOD is a Fe-SOD.

REGISTRY NUMBERS: 9054-89-1: SUPEROXIDE DISMUTASE; 9001-05-2: CATALASE;
57-12-5: CYANIDE; 14343-69-2: AZIDE
DESCRIPTORS:
MAJOR CONCEPTS: Bacteriology; Enzymology (Biochemistry and Molecular
Biophysics)
BIOSYSTEMATIC NAMES: Vibrionaceae-- Facultatively Anaerobic
Gram-Negative Rods, Eubacteria, Bacteria, Microorganisms
ORGANISMS: Photobacterium phosphoreum var. K (Vibrionaceae); Vibrio sp.
(Vibrionaceae)
BIOSYSTEMATIC CLASSIFICATION (SUPER TAXA): Bacteria; Eubacteria;
Microorganisms
CHEMICALS & BIOCHEMICALS: azide ; catalase--hemoprotein, isozyme;
cyanide; superoxide dismutase {SOD}--isozyme; 3-amino,1,2,4-triazole
MISCELLANEOUS TERMS: bioluminescence transmission; pH
CONCEPT CODES:
30000 Bacteriology, General and Systematic
10060 Biochemical Studies-General
10802 Enzymes-General and Comparative Studies; Coenzymes
BIOSYSTEMATIC CODES:
06704 Vibrionaceae (1992-)

6/9/12 (Item 12 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

11958317 BIOSIS NO.: 199900204426
Bacterial proteins carrying twin-R signal peptides are specifically
targeted by the DELTApH-dependent transport machinery of the thylakoid
membrane system.
AUTHOR: Halbig Dirk; Hou Bo; Freudl Roland; Sprenger Georg A; Kloesgen Ralf
Bernd(a)
AUTHOR ADDRESS: (a)Institut fuer Pflanzen- und Zellphysiologie,
Martin-Luther-Universitaet Halle-Wittenberg, Am Kir**Germany
JOURNAL: FEBS Letters 447 (1):p95-98 March 19, 1999
ISSN: 0014-5793
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English
SUMMARY LANGUAGE: English

ABSTRACT: Glucose-fructose oxidoreductase (GFOR), a periplasmic protein of
Zymomonas mobilis, is synthesized as a precursor polypeptide with a
twin-R signal peptide for Sec-independent protein export in bacteria. In
higher plant chloroplasts, twin-R signal peptides are specific targeting
signals for the Sec-independent DELTApH pathway of the thylakoid membrane
system. In agreement with the assumed common phylogenetic origin of the
two protein transport mechanisms, GFOR can be efficiently translocated by
the DELTApH-dependent pathway when analyzed with isolated thylakoid
membranes. Transport is sensitive to the ionophore nigericin and competes
with specific substrates for the DELTApH-dependent transport route. In
contrast, neither sodium azide nor enzymatic destruction of the
nucleoside triphosphates in the assays affects thylakoid transport of
GFOR indicating that the Sec apparatus is not involved in this process.
Mutagenesis of the twin-R motif in the GFOR signal peptide prevents
membrane translocation of the protein emphasizing the importance of these
residues for the transport process.

REGISTRY NUMBERS: 9035-73-8: OXIDASE
DESCRIPTORS:
MAJOR CONCEPTS: Biochemistry and Molecular Biophysics; Membranes (Cell
Biology)
BIOSYSTEMATIC NAMES: Facultatively Anaerobic Gram-Negative Rods--
Eubacteria, Bacteria, Microorganisms; Leguminosae--Dicotyledones,
Angiospermae, Spermatophyta, Plantae
ORGANISMS: Pisum sativum {pea} (Leguminosae); Zymomonas mobilis (
Facultatively Anaerobic Gram-Negative Rods)
ORGANISMS: PARTS ETC: chloroplast; thylakoid membrane--
delta-pH-dependent transport machinery
BIOSYSTEMATIC CLASSIFICATION (SUPER TAXA): Angiosperms; Bacteria; Dicots;
Eubacteria; Microorganisms; Plants; Spermatophytes; Vascular Plants
CHEMICALS & BIOCHEMICALS: glucose-fructose oxidase--periplasmic protein
; twin-R signal peptide-containing bacterial proteins--targeting
CONCEPT CODES:
10508 Biophysics-Membrane Phenomena
02504 Cytology and Cytochemistry-Plant
10064 Biochemical Studies-Proteins, Peptides and Amino Acids
31000 Physiology and Biochemistry of Bacteria
51520 Plant Physiology, Biochemistry and

Biophysics-Translocation,
Accumulation
10808 Enzymes-Physiological Studies
10506 Biophysics-Molecular Properties and Macromolecules
BIOSYSTEMATIC CODES:
06700 Facultatively Anaerobic Gram-Negative Rods (1992-)
26260 Leguminosae

6/9/13 (Item 13 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

11957662 BIOSIS NO.: 199900203771
Purification and cloning of a thermostable manganese catalase from a
thermophilic bacterium.
AUTHOR: Kagawa Masayuki; Murakoshi Noriyuki; Nishikawa Yasushi; Matsumoto
Gen; Kurata Youko; Mizobata Tomohiro; Kawata Yasushi; Nagai Jun(a)
AUTHOR ADDRESS: (a)Dep. Biotechnol., Fac. Eng., Tottori Univ.,
Koyama-Minami, Tottori 680-8552**Japan
JOURNAL: Archives of Biochemistry and Biophysics 362 (2):p346-355 Feb. 15,
1999
ISSN: 0003-9861
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: We have purified a heat-stable catalase from a thermophilic
bacterium, Thermus species strain YS 8-13. The enzyme was purified
160-fold from crude cellular extracts and possessed a specific activity
of 8000 units/mg at 65degree C. The purified enzyme displayed the highest
activity at pH 7 to 10 and temperatures around 85degree C. The catalase
was determined to be a manganese catalase, based on results from atomic
absorption spectra and inhibition experiments using sodium azide . The
enzyme was composed of six identical subunits of molecular weight 36,000.
Amino acid sequences determined from the purified protein were used to
design oligonucleotide primers, which were in turn used to clone the
coding gene. The nucleotide sequence of a 1.4-kb fragment of Thermus sp.
YS 8-13 genomic DNA containing a 909-bp open reading frame was
determined. The gene encoded a 302-residue polypeptide of deduced
molecular weight 33,303. The deduced amino acid sequence displayed a
region-specific homology with the sequences of the manganese catalase
from a mesophilic organism, Lactobacillus plantarum.

DESCRIPTORS:
MAJOR CONCEPTS: Enzymology (Biochemistry and Molecular Biophysics);
Molecular Genetics (Biochemistry and Molecular Biophysics)
BIOSYSTEMATIC NAMES: Enterobacteriaceae--Facultatively Anaerobic
Gram-Negative Rods, Eubacteria, Bacteria, Microorganisms; Gram-Negative
Aerobic Rods and Cocci--Eubacteria, Bacteria, Microorganisms
ORGANISMS: Escherichia coli (Enterobacteriaceae)--gene expression vector;
Thermus sp. (Gram-Negative Aerobic Rods and Cocci)--strain-YS 8-13,
thermostabile
BIOSYSTEMATIC CLASSIFICATION (SUPER TAXA): Bacteria; Eubacteria;
Microorganisms
CHEMICALS & BIOCHEMICALS: manganese catalase--molecular cloning,
purification
MOLECULAR SEQUENCE DATABANK NUMBER: AB008786--DDBJ, nucleotide sequence
METHODS & EQUIPMENT: atomic absorption spectroscopy--analytical method;
SDS-PAGE {SDS-polyacrylamide gel electrophoresis}--analytical method
CONCEPT CODES:
31500 Genetics of Bacteria and Viruses
10060 Biochemical Studies-General
10502 Biophysics-General Biophysical Studies
10802 Enzymes-General and Comparative Studies; Coenzymes
30000 Bacteriology, General and Systematic
BIOSYSTEMATIC CODES:
06500 Gram-Negative Aerobic Rods and Cocci (1992-)
06702 Enterobacteriaceae (1992-)

6/9/1 (Item 1 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

12399859 BIOSIS NO.: 200000153361
Characterization of recombinant fatty acid alpha-hydroxylating cytochrome
P450 (CYP152B1).
AUTHOR: Sumimoto Tatsuo(a)
AUTHOR ADDRESS: (a)Department of Molecular Regulation, Osaka City
University Medical School, Osaka**Japan
JOURNAL: Journal of the Osaka City Medical Center. 48 (3-4):p485-503 Dec.,
1999
ISSN: 0386-4103

DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: Japanese; Non-English
SUMMARY LANGUAGE: English; Japanese

ABSTRACT: Fatty acid alpha-hydroxylase catalyzes the initial reaction in fatty acid alpha-oxidation to produce 2-hydroxy fatty acid as an intermediate. alpha-Oxidation activity has been detected in various species. However, the enzymatic properties of fatty acid alpha-hydroxylase have not been characterized using a purified enzyme.

The fatty acid alpha-hydroxylase gene was recently cloned from Sphingomonas paucimobilis, a bacterium with a large amount of 2-hydroxy fatty acid, and this bacterial alpha-hydroxylase was shown to be a novel member of the cytochrome P450 superfamily (CYP152B1). In this study, I characterized the recombinant fatty acid alpha-hydroxylase purified from Eschericia coli. The recombinant fatty acid alpha-hydroxylase, which was used in a previous study, was truncated by 18 amino acids at the N terminus and contained a 10-amino-acid sequence from lacZ. Thus, I reconstructed the expression vector containing the full length of the fatty acid alpha-hydroxylase gene and purified these two recombinant forms of the alpha-hydroxylase to compare their properties. Two recombinant forms of the alpha-hydroxylase were similar in spectral properties, Km value for hydrogen peroxide and effects of various inhibitors. However, the Km value of the recombinant form containing the full length of alpha-hydroxylase was similar to that of the native enzyme from S. paucimobilis, but the Km value of the truncated form was 6-fold greater than that of the full-form. I therefore characterized the recombinant enzyme containing the full length of alpha-hydroxylase in the experiments described below, since this recombinant enzyme appeared to have properties similar to those of the native enzyme. The Km values for myristic acid and hydrogen peroxide were 41.4 muM and 65 muM, respectively. In contrast to hydrogen peroxide, additions of cumene hydroperoxide, t-butyl hydroperoxide, or t-butyl peroxybenzonate to reaction mixture did not affect alpha-hydroxylation activity. The recombinant alpha-hydroxylase had a spectrum with absorption peaks at 568, 536, 418 and 362 nm in the oxidized form. The spectrum of the dithionite-reduced form had absorption peaks at 545 and 405 nm. The CO difference spectrum of the recombinant alpha-hydroxylase was characteristic of P450, except its peak was at 445 nm. Of the inhibitors tested, SKF-525A, a cytochrome P450 inhibitor, markedly inhibited alpha-hydroxylation activity, but carbon monoxide did not. Potassium cyanide and sodium azide with inhibited alpha-hydroxylation activity.

The recombinant fatty acid alpha-hydroxylase metabolized fatty acids with carbon chain lengths of 11 to 18 in the presence of hydrogen peroxide. Tridecanic acid, myristic acid and pentadecanoic acid were well metabolized by this enzyme with Vmax/Km values of 0.099, 0.120 and 0.137 nM-1min-1, respectively. However, the turnover rate was dramatically decreased when undecanoic acid was used as a substrate, and capric acid was not metabolized. The recombinant alpha-hydroxylase metabolized monounsaturated fatty acids, methyl-branched fatty acids, polyunsaturated fatty acids and omega-hydroxy fatty acids. The reaction products when phytanic acid, arachidonic acid and 16-hydroxypalmitic acid were used as the substrates were determined by gas chromatography-mass spectrometry to be the corresponding 2-hydroxy fatty acids. The recombinant alpha-hydroxylase did not metabolize methyl myristate, myristoyl coenzyme A, N-myristoylshingosine, tetradecane, tetradecanal, 1-tetradecanol, benzphetamine, 7-ethoxycoumarin and 7-ethoxyresorufin as substrates.

These findings suggest that the alpha-hydroxylase recognized the carboxyl moiety, the alkyl chain, and the structure of omega-end of the fatty acid substrates and regioselectively hydroxylated carbon at 2 position of the substrates.

REGISTRY NUMBERS: 9035-51-2: CYTOCHROME P450; 7722-84-1: HYDROGEN PEROXIDE; 544-63-8: MYRISTIC ACID
DESCRIPTORS:
MAJOR CONCEPTS: Biochemistry and Molecular Biophysics

BIOSYSTEMATIC NAMES: Enterobacteriaceae--Facultatively Anaerobic
Gram-Negative Rods, Eubacteria, Bacteria, Microorganisms;
Pseudomonadaceae--Gram-Negative Aerobic Rods and Cocci, Eubacteria,
Bacteria, Microorganisms
ORGANISMS: Escherichia coli (Enterobacteriaceae); Sphingomonas
paucimobilis (Pseudomonadaceae)
BIOSYSTEMATIC CLASSIFICATION (SUPER TAXA): Bacteria; Eubacteria;
Microorganisms
CHEMICALS & BIOCHEMICALS: cytochrome P450--characterization,
recombinant fatty acid alpha-hydroxylase; hydrogen peroxide; myristic
acid
METHODS & EQUIPMENT: spectrophotometry--analytical method
CONCEPT CODES:
10066 Biochemical Studies-Lipids
10504 Biophysics-General Biophysical Techniques
10802 Enzymes-General and Comparative Studies; Coenzymes
31000 Physiology and Biochemistry of Bacteria
BIOSYSTEMATIC CODES:
06508 Pseudomonadaceae (1992-)
06702 Enterobacteriaceae (1992-)
?logoff hold
24aug02 10:45:18 User228206 Session D1842.3
$4.48 0.799 DialUnits File5
$66.50 38 Type(s) in Format 9
$66.50 38 Types
$70.98 Estimated cost File5
$0.09 0.005 DialUnits File34
$9.70 2 Type(s) in Format 9
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$0.01 Estimated cost File94
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$6.60 4 Types
$8.21 Estimated cost File144
$0.07 0.016 DialUnits File149
$6.90 2 Type(s) in Format 9
$6.90 2 Types
$6.97 Estimated cost File149
$1.43 0.447 DialUnits File155
$1.47 7 Type(s) in Format 9
$1.47 7 Types
$2.90 Estimated cost File155
$0.00 0.002 DialUnits File156
$0.00 Estimated cost File156
$0.01 0.002 DialUnits File159
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$0.02 0.002 DialUnits File172
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$0.01 0.002 DialUnits File467
$0.01 Estimated cost File467
OneSearch, 28 files, 2.087 DialUnits FileOS
$0.21 TELNET
$118.73 Estimated cost this search
$118.73 Estimated total session cost 2.087 DialUnits

Status: Signed Off. (1 minutes)

L6: Entry 123 of 176 File: USPT Apr 12, 1983

DOCUMENT-IDENTIFIER: US 4379847 A
TITLE: Suspending medium for immunologic reactions

CLAIMS:

5. The suspending medium of claim 1 wherein the osmolality is from about 250 mOsm/kg. H.sub.2 O to about 400 mOsm/kg. H.sub.2 O, and the salt is sodium chloride, potassium chloride, or sodium azide.

6. The suspending medium of claim 3 wherein the osmolality is from about 250 mOsm/kg. H.sub.2 O to about 400 mOsm/kg. H.sub.2 O, the gelatin Bloom rating times concentration is from about 45 to about 90, and the salt is sodium chloride, potassium chloride, or sodium azide.

13. The suspending medium of claim 1 wherein the salt is sodium azide.

14. The suspending medium of claim 3 wherein the salt is sodium azide.

15. The suspending medium of claim 8 wherein the salt is sodium azide.

21. The suspending medium of claim 20 wherein the antibiotic is neomycin or chloramphenicol, or the bacteriostat is thimerosal, phenylmercuric acetate, or sodium azide.

L11: Entry 8 of 9 File: USPT Jul 9, 1985

DOCUMENT-IDENTIFIER: US 4528199 A
TITLE: Silage production from fermentable forages

Detailed Description Text (31):
Samples were plated in duplicate on selective and nonselective agars. Trypticase soy broth plus agar 1.5% (TSB+A) (Difco, Detroit, MI) was used as a general plating medium to recover facultative anaerobic or microaerophilic bacteria. Lactobacillus selective agar (LBS, Baltimore Biological Laboratory, MD) was used to recover Lactobaccilli. Azide dextrose broth+1.5% agar (AZD) was used to recover lactic acid cocci which were predominately streptococci. Plates were incubated in a reduced oxygen (15% CO.sub.2, 85% air) atmosphere at 32.degree. C. for 1 week before colonies were enumerated. Yeasts and molds were enumerated on the rose bengal chlortetracycline agar (YM) described by Jarvis, J. Appl. Bacteriol., 36, 723 (1973). Coliforms were enumerated on violet red bile (VRB) agar. The YM was incubated aerobically at 30.degree. C. for 1 wk and VRB for 48 h before enumeration of colonies.

Detailed Description Text (38):
Facultative anaerobic bacteria were increased (P<0.05) by day 32 for alfalfa, corn, sorghum, and wheat silages with addition of L. plantarum 2B (FIGS. 3, 4, Table 4). Populations in corn and sorghum silages reached a maximum after 1 day and then declined. Populations in alfalfa and wheat silages reached maximum later in fermentation (FIG. 4). Total lactobacilli counts were increased (P<0.05) by addition of L. plantarum 2B in alfalfa, wheat, and sorghum silages but not in corn silage (FIGS. 5, 6, Table 4). Initial populations of lactobacilli were much lower (10.sup.3 to 10.sup.5 /g) in control silages of alfalfa and wheat (FIG. 6) than inoculated silages. Initial populations of lactobacilli were 10.sup.6 to 10.sup.7 /g in corn and sorghum silages which was similar to inoculum. Populations of lactic acid cocci recovered on azide dextrose agar were similar in all silages (FIGS. 7, 8, Table 4). Yeast and mold counts were lowered (P<0.05) by addition of L. plantarum 2B in alfalfa and wheat silages (FIGS. 9, 10, Table 4), but it did not have a significant effect on corn or sorghum silages.

Detailed Description Paragraph Table (6):
TABLE 4

Effect of inoculation of alfalfa, corn, sorghum, and wheat silages with Lactobacillus plantarum 2B on mean pH and microbial populations (log.sub.10 cell number/g silage) recovered on four different agar media. Agar medium.sup.a Silage Treatment pH Axide dextrose LBS TSB + A YM

Alfalfa Control 4.71 8.35 8.41 9.09 4.76 L. plantarum 4.36.sup.bc 7.89 9.12.sup.c 9.46.sup.bc 4.25.sup.bc Corn Control 3.74 8.05 8.25 8.59 5.14 L. plantarum 3.77 8.22 8.52 8.97.sup.bc 5.39 Sorghum Control 3.74 7.95 7.83 8.35 5.57 L. plantarum 3.79 8.66.sup.bc 8.45.sup.bc 8.97.sup.bc 5.39 Wheat Control 4.62 8.08 7.35 8.63 5.35 L. plantarum 4.14.sup.bc 8.03 8.96.sup.bc 9.28.sup.bc 4.45.sup.bc

_____ .sup.a Agar medium. Azide dextrose for lactic acid cocci, LBS for lactobacilli, TSB + A for total facultative anaerobes, YM for yeasts and molds. .sup.b,c Means followed by b differed (P < .05) from control silages as determined by a Ducan' s tests of treatment means; when followed by c, means differed (P < .05) from control silages using linear regression models determined over the 33 day fermentation period. The standared erro of the means of duplicate determinations was azide dextrose .288, LBS .189, TSB + A .288, YM .288, pH .075.

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L19: Entry 9 of 11 File: USPT Aug 30, 1994

DOCUMENT-IDENTIFIER: US 5342763 A
TITLE: Method for producing polypeptide via bacterial fermentation

Abstract Text (1):
A process for producing a polypeptide of interest from fermentation of bacterial host cells comprising nucleic acid encoding the polypeptide is provided. In this method, the host cells employed have an inactivated electron transport chain. Further provided is a method for determining if a particular bacterial cell culture has a propensity for dissolved oxygen instability when fermented on a large scale.

Brief Summary Text (21):
For fed-batch fermentations in which the increasing broth volume often significantly changes the geometric relationship between the agitator impellers and the liquid volume, and in larger fermentors, i.e., those having at least approximately 1000 liters of capacity, preferably about 1000 to 100,000 liters of capacity, the preferred solution to the problem addressed by this invention to allow the most efficient use of the fermentation vessel is to mutate the host organism to inactivate a key element of one of the respiration pathways (e.g., cytochrome d oxidase complex, cytochrome o oxidase complex, menaquinone, or one of the NADH dehydrogenases) so that the organism can no longer switch between the two electron transport pathways.

Brief Summary Text (22):
Specifically, the present invention provides a process for producing a polypeptide of interest from a fermentation of bacterial host cells comprising nucleic acid encoding the polypeptide which method comprises conducting the fermentation using bacterial host cells having an impaired electron transport chain, i.e., an inactivation in one of their respiratory chains.

Drawing Description Text (42):
A bacterial organism with an "impaired" or "inactivated" electron transport chain refers to bacteria that are mutated so as to render inactive or disable at least one, but not all, of the electron carriers constituting its electron transport chains. This mutation may be by way of deletion of the genetic component representing an electron carrier, or alternatively, by alterations in the nucleotides of the genetic component such that it no longer functions in the way defined above. Thus, for example, if the genetic component is cytochrome o or d oxidase, the organism may produce the cytochrome o oxidase gene product but not the cytochrome d oxidase gene product, or the cytochrome d oxidase gene product but not the cytochrome o oxidase gene product. The preferred bacterial organism has an inactivated cytochrome d or o oxidase gene, more preferably an inactivated cytochrome o oxidase gene, and most preferably lacks the latter gene.

Drawing Description Text (45):

For purposes of this invention, an altered host strain contains one or more nucleotide mutations within its electron transport chains, preferably its cytochrome o oxidase or cytochrome d oxidase complex gene, so that any one or more, but not all, of the electron carrier genes in the electron transport chains is inactivated. The strain is preferably an E. coli strain. Such strain variants are suitably prepared by introducing appropriate nucleotide changes into the bacterial strain DNA. The strain variants include, for example, deletions from, or insertions or substitutions of, nucleotides within the nucleic acid sequence of the native electron transport chain gene sufficient to prevent the gene from allowing the strain to switch from one aerobic respiratory chain to the other under conditions favoring DO.sub.2 instability. Such genes can be readily identified by the methods outlined in Example III below. Any combination of deletion, insertion, and substitution can be made to arrive at the final strain, provided that the final strain possesses the desired characteristics.

Drawing Description Text (83):
The bacterial host cells used for expressing the vectors encoding the polypeptide of interest are those that contain at least one operable electron carrier component that mediates the electron transport chain, so that the respiration pathway of the cells is not totally impaired. In the method herein, a suitable strain utilized for this purpose is typically one that is mutated such that one, but not all, of its native electron carriers is inactivated. Preferably, this inactivation is achieved by replacement of the native electron carrier gene with a variant electron carrier gene that is homologous to the native electron carrier gene normally present in the host cells.

Drawing Description Text (84):
All bacteria, including both archaebacteria and eubacteria, generally have more than one terminal oxidase (Anraku and Gennis, supra), and thus all except obligate anaerobes are potentially susceptible to DO.sub.2 instabilities upon culturing. Suitable bacteria for this purpose include aerobic and facultative anaerobic bacteria, whether archaebacteria and eubacteria, especially eubacteria, and most preferably Enterobacteriaceae. Examples of useful bacteria include Escherichia, Enterobacter, Azotobacter, Erwinia, Bacillus, Pseudomonas, Klebsiella, Proteus, Salmonella, Serratia, Shigella, Rhizobia, Vitreoscilla, and Paracoccus. Suitable E. coli hosts include E. coli W3110 (ATCC 27,325), E. coli 294 (ATCC 31, 446), E. coli B, and E. coli X1776 (ATCC 31,537). These examples are illustrative rather than limiting. Mutant cells of any of the above-mentioned bacteria may also be employed. It is, of course, necessary to select the appropriate bacteria taking into consideration replicability of the replicon in the cells of a bacterium. For example, E. coli, Serratia, or Salmonella species can be suitably used as the host when well known plasmids such as pBR322, pBR325, pACYA177, or pKN410 are used to supply the replicon.

Other Reference Publication (14):
Harrison et al., "The Effect of Growth Conditions on Respiratory Activity and Growth Efficiency in Facultative Anerobes Grown in Chemostat Culture" J. of General Microbiology 68:35-43 (1971).

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L22: Entry 1 of 2 File: USPT Aug 4, 1998

DOCUMENT-IDENTIFIER: US 5789191 A
TITLE: Method of detecting and counting microorganisms

CLAIMS:

8. The method according to claim 1, wherein the selective medium is for testing anaerobic microorganisms and consists of 35 to 50 parts by weight of a customary base medium and one or more selectors selected from the group consisting of sodium thioglycolate, NaCl, L-cysteine, HCl, resazurin and NaHCO.sub.3.

13. The method according to claim 1, wherein the selective medium is for testing enterococci and consists of 30 to 60 parts by weight of a customary base medium and one or more selectors selected from the group consisting of sodium citrate, sodium azide, thallium acetate and 2,3,5-triphenyltetrazole.

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End of Result Set

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L25: Entry 18 of 18 File: DWPI Dec 25, 1984

DERWENT-ACC-NO: 1985-061539
DERWENT-WEEK: 198510

TITLE: Selection of amylolytic enzyme microorganisms - by anaerobic incubation and detection of hydrolysed starch zone around colonies

INVENTOR: HORWATH, R O

PATENT-ASSIGNEE: NABISCO BRANDS INC (NATY)

PRIORITY-DATA: 1983US-0480430 (March 30, 1983)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
US 4490466 A	December 25, 1984		004	

APPLICATION-DATA:

PUB-NO	APPL-DATE	APPL-NO	DESCRIPTOR
US 4490466A	March 30, 1983	1983US-0480430	

INT-CL (IPC): C12Q 1/40

ABSTRACTED-PUB-NO: US 4490466A
BASIC-ABSTRACT:

Selection and screening of micro-orqanisms for the prodn. of amylolytic enzymes comprises (a) forming a screening plate comprising a suspn. of the micro-orqanism on a solid medium promoting the growth of the micro-organisms and the synthesis of amylolytic enzymes; (b) incubating the medium anaerobically for sufficient ime for qrowth of a screenable sub-popula-tion; and (c) identification in situ of the colonies producing the enzymes by detection of a zone of hydrolysed starch surrounding each colony.

USE/ADVANTAGE - The procedure is rapid and efficient for the combined selection and screening of micro-orqanisms for prodn. of the enzymes. Alpha-amylases having a low Ca requirement may be distinguished from these with a hiqh requirement by inclusion of a Ca-chelating agent in the medium.

ABSTRACTED-PUB-NO: US 4490466A
EQUIVALENT-ABSTRACTS:

DERWENT-CLASS: D16
CPI-CODES: D05-A02; D05-H04; D05-H05;

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Generate Collection Print

L30: Entry 6 of 28 File: USPT Dec 5, 2000

DOCUMENT-IDENTIFIER: US 6156329 A
TITLE: Stripped spent silver catalysts and novel uses thereof

Brief Summary Text (56):
Escherichia coli (ATTC No. 11229), a gram negative bacterium, was subcultured onto standard plate count agar, and Enterococcus faecium (ATTC No. 6569), a gram positive bacterium, was subcultured onto bile exculin azide agar the day before the biocide test. On the test day the cells were harvested by removing the growth from the agar surface using five milliliters of phosphate buffered water. The cells were centrifuged in sterile tubes to remove any media debris and the supernatant was transferred to a sterile container. The cell concentration was determined by the percent light transmission using a Genesys 5 spectrophotometer at 530 nm and compared with the laboratory database. The E.coli transmission was adjusted to 88% and the E. faecium to 85% to have approximately 2.times.10.sup.8 cells per milliliter.

L30: Entry 24 of 28 File: USPT Jul 30, 1985

DOCUMENT-IDENTIFIER: US 4532206 A
TITLE: .beta.-Streptococcus selective medium

Brief Summary Text (11):
The above objects and advantages of the present invention for the growth of .beta.-hemolytic streptococci only, without the growth of enterococci and the other non-.beta.-hemolytic streptococci, is based on the concept of the incorporation of pullulan into the medium, together with other essential components, as described below. This differentiation or split is achieved by the ability of all .beta.-hemolytic streptococci tested to produce acid from the unusual substrate pullulan. Pullulan is a commercially available carbohydrate that is used in the present invention to split out the non-.beta.-hemolytic streptococci due to their inability to produce acid from it. Most media presently available select for the growth of the enterococci. These include SF Broth, Enterococcus Confirmatory Broth, Ethyl Violet Azide Broth, Mitis Salivarius Agar, and others.

WEST Search History

DATE: Friday, August 23, 2002

Set Name sid	Query by side	Hit Count	Set Name result set
DB=USPT,PGPB,JPAB,EPAB,DWPI,TDBD; PLUR=YES; OP=AND			
L1	($azide or azid$).clm.	7932	L1
L2	(media or medium or agar or agarous or broth or bhi or brucella or cdc or nutrient or schaedler or thioglycolate or trypticase or growth).clm.	214767	L2
L3	(microaerophil$ or micro-aerofilic or anaerob$ or facultativ$).clm.	2103	L3
L4	L3 and l1	16	L4
L5	l1 and l2	749	L5
L6	l1 same l2	176	L6
L7	l3 same l2 not l6	403	L7
L8	L7 not l4	403	L8
L9	L8	403	L9
L10	(microaerophil$ or micro-aerofilic or facultativ$) same anaerob$	1457	L10
L11	L10 same azide	9	L11
L12	(oxygen near3 (sensitive or toxic)) or anaerob$	33462	L12
L13	L12 same ($azide or azid$)	86	L13
L14	electron near3 transport$	8031	L14
L15	antagonist or inhibit$ or block$ or inactiva$ or modulat$	2770750	L15

L16	L15 same l14	985	L16
L17	L16 same (microaerophil$ or micro-aerofilic or facultativ$)	0	L17
L18	L16 same (microaerophil$ or micro-aerofilic or facultativ$ or micro-aerophil$)	0	L18
L19	L16 and (microaerophil$ or micro-aerofilic or facultativ$ or micro-aerophil$)	11	L19
L20	select$ near5 anaerob$	489	L20
L21	L20 same azide	1	L21
L22	L20.clm. and $azide.clm.	2	L22
L23	select$ near5 slow$ near5 (growing or grows or replication or replicating or divid or divids or dividing)	89	L23
L24	L23 and (mix$ same cultur$)	38	L24
L25	l20.ti.	18	L25
L26	faciltative or microaerophilic or micro-aerophilic or microaerophiles	498	L26
L27	L26.ti.	15	L27
L28	L27 and azide	0	L28
L29	l2 same azide.clm.	70	L29
L30	azide near3 (agar or agarose or broth)	28	L30

END OF SEARCH HISTORY

WEST

Generate Collection Print

L6: Entry 29 of 176 File: USPT Nov 28, 2000

DOCUMENT-IDENTIFIER: US 6153400 A
TITLE: Device and method for microbial antibiotic susceptibility testing

CLAIMS:

34. The method according to claim 7, wherein said selective media comprises one or more of, columbia CNA blood, azide blood agar, chocolate selective, Brucella blood, blood SxT, Strep selective I & II, PEA, Bile Esculin agar, Clostridium difficile agar, skirrow, CCFA, CLED, Pseudomonas cepacia agar, SxT blood agar, TCBS agar, CIN, Moraxella catarrhalis media, and charcoal selective.

WEST

☐ Generate Collection | Print

L6: Entry 21 of 176 File: USPT Mar 12, 2002

US-PAT-NO: 6355449
DOCUMENT-IDENTIFIER: US 6355449 B1

TITLE: Method and medium for detecting vancomycin-resistant enterococcus

DATE-ISSUED: March 12, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Chen; Chung-Ming	Falmouth	ME		
Edberg; Stephen C.	Orange	CT		

US-CL-CURRENT: 435/34; 435/16, 435/29, 435/36, 435/38, 435/39, 435/61, 435/7.2, 435/7.36, 435/885

CLAIMS:

What is claimed is:

1. A medium for detecting vancomycin-resistant Enterococci in a sample form a rectal swab, peri-rectal swab, or stool sample comprising:

vancomycin in an amount sufficient to suppress the growth of vancomycin sensitive Enterococci;

a first nutrient indicator which is a substrate for a first bacterial enzyme and provides a first detectable signal when cleaved by the first bacterial enzyme wherein the first nutrient indicator is a substrate for--.beta.-glucosidase;

a second nutrient indicator which is a substrate for a second bacterial enzyme and provides an intermediate molecule when cleaved by the second bacterial enzyme, and the intermediate molecule provides the second detectable signal upon reacting with a developing agent, wherein the second detectable signal is distinct from the first detectable signal wherein the second nutrient indicator is a substrate for pyrrolidonyl arylamidase;

an effective amount of one or more selective agents active to prevent or inhibit the growth of microorganisms other than Enterococci when a sample from a rectal swab, peri-rectal swab, or stool sample is introduced into the medium wherein the one or more selective agents are selected form the group consisting of: amikacin sulfate, polymyxin B, bacitracin, clindamycin, cefotaxime, amphotericin B, sodium azide, thallium acetate, nalixidic acid, enoxacin, cinoxacin,. ofloxacin, norfloxacin, cefotaxime, gentamycin, neomycin, polymyxin B, colistin, and bile salts.

2. The medium of claim 1 wherein the first nutrient indicator produces a detectable color after being cleaved by .beta.-glucosidase.

3. The medium of claim 1 wherein the first nutrient indicator is selected from the group consisting of resofuran-.beta.-glucopyranoside, o-nitrophenyl-.beta.-glycopyranoside, p-nitrophenyl-.beta.-D-glycopyranoside, 5-bromo-4chloro-3-indoxyl-.beta.-D-glucopyranoside, 6-bromo-2-naphtyl-.beta.-D-glycopyranoside, Rose-.beta.-D-glycopyranoside, VQM-Glc(2-{2-[4-(.beta.-D-glucopyranosyloxy)3-methoxyl]vinyl)-1-methyl-qui nolinium iodide, VBZTM-Gluc(2-{2-[4-(.beta.-D-glucopyranosyloxy)3-methoxylphenyl]vinyl}-3-m ethylbenzothiazolium iodide, and 4-methylumbelliferyl-.beta.-D-glycopyranoside.

4. The medium of claim 1 wherein the second nutrient indicator is selected from the group consisting of L-pyroglutamic acid p-nitroanilide, L-pyroglutamic acid 7-amido-4-methyl-coumarin, and pyroglutamic acid .beta.-naphtylamide.

5. The medium of claim 1 wherein the intermediate molecule alters the color of the medium upon reacting with a color developing agent.

6. The medium of claim 5 wherein the second nutrient indicator is pyroglutamic acid-.beta.-naphtylamide.

7. The medium of claim 6 wherein the developing agent is p-dimethylamino-cinnamaldehyde.

8. The medium of claim 2 wherein the first nutrient indicator is o-nitrophenyl-.beta.-D-glucopyranoside.

9. The medium of claim 1 further comprising one or more inducers of enzyme activity for .beta.-D-glucopyranoside.

10. The medium of claim 1 in a liquid form or a gel form.

11. The medium of claim 9 wherein the one or more inducers of enzyme activity are selected form the group consisting of: isopropyl-.beta.-D-thiogalactoside (IPTG), ethyl-.beta.-D-thioglucoside, L-pyroglutamamide, L-pyroglutamic acid, and pyroglutamic acid pentachlorophenyl ester.

12. The medium of claim 1 wherein the first nutrient indicator produces a color in the visual range when cleaved by an enzyme, and the second nutrient indicator produces a fluorescent molecule when cleaved by an enzyme.

13. The medium of claim 12 wherein the medium further comprises a sample from a wound swab, a urine specimen, or a swab from a utensil or equipment surface.

WEST

Generate Collection Print

L6: Entry 30 of 176 File: USPT Nov 14, 2000

DOCUMENT-IDENTIFIER: US 6146840 A
TITLE: Simultaneous enumeration of E. coli and total coliforms

CLAIMS:

5. A method according to claim 2, wherein, prior to said detecting step said bacterial colony is contacted with a reagent which limits bacterial growth or incubated under conditions which limit bacterial growth, said reagent being at least one of azide, cyanide, a semitoxic dye, antibiotic, urea and guanidine, said conditions being starvation for at least one of a nitrogen source, phosphate and salts, and the intensity of said lower intensity first reaction product signal at said second region is further reduced as compared with said first region.

WEST

Generate Collection Print

L4: Entry 6 of 16 File: USPT Aug 4, 1998

US-PAT-NO: 5789191
DOCUMENT-IDENTIFIER: US 5789191 A

TITLE: Method of detecting and counting microorganisms

DATE-ISSUED: August 4, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Mayer; Bianca	Hamburg			DE
Sauermann; Gerhard	Wiemersdorf			DE
Traupe; Bernd	Hamburg			DE
Wolf; Florian	Hamburg			DE

US-CL-CURRENT: 435/39; 435/254.2, 435/254.22, 435/255.1, 435/29, 435/30, 435/32, 435/34, 435/36, 435/38, 435/848, 435/849, 435/882, 435/883, 435/884, 435/921, 435/922, 435/923

CLAIMS:

We claim:

1. A cosmetic or dermatological method for detecting or selectively quantifying nonpathological individual microorganism or whole groups of microorganisms or a pathological microorganism or whole groups of microorganisms which cause cosmetic disorders or dermatological diseases wherein said microorganisms are present on human or animal skin, said method comprises

scraping a defined area on said skin to remove a sample of microflora;

treating the sample with a deinhibiting medium;

adding the sample obtained above into a culture medium which exhibits favorable growth conditions for the microorganisms or group of microorganisms but unfavorable growth for other microorganisms present in the microflora so that a selective culture is produced;

incubating the resulting selective culture for a time sufficient for the microorganisms to multiply and to produce metabolic products;

collecting the metabolic products either by collecting the culture medium itself or by collecting the products in a test vessel containing an indicator medium; and

US Pat 5789,191

measuring, after calibration the concentration of metabolic products by measuring the change in the alternating current in the culture medium or the indicator medium and determining the number of microorganisms in the selective medium by arithmetic method.

2. The method according to claim 1, wherein the metabolic product is CO.sub.2.

3. The method according to claim 1, wherein the skin is first rinsed for a defined period of time with an aqueous solution of a surface-active agent which is buffered to a pH between 5.0 to 8.0 and then scraped with a spatula.

4. The method according to claim 3, wherein the spatula is coated with a synthetic material.

5. The method according to claim 4, wherein the synthetic material is Teflon.

6. The method according to claim 1, wherein the selective medium is for testing Staphylococci and consists of 40 to 50 parts by weight of a customary base medium and one or more selectors selected from the group consisting of sodium pyruvate, glycin, KSCN, NaH.sub.2 PO.sub.4, Na.sub.2 HPO.sub.8, LiCl, aztreonam and linolenic acid.

7. The method according to claim 1, wherein the selective medium is for testing Propioni bacterium spec. and consists of 35 to 50 parts by weight of a customary base medium and one or more selectors selected from the group consisting of sodium thioglycolate, NaCl, L-cysteine, HCl, resazurin and NaHCO.sub.3 and phosphomycin.

8. The method according to claim 1, wherein the selective medium is for testing anaerobic microorganisms and consists of 35 to 50 parts by weight of a customary base medium and one or more selectors selected from the group consisting of sodium thioglycolate, NaCl, L-cysteine, HCl, resazurin and NaHCO.sub.3.

9. The method according to claim 1, wherein the selective medium is for testing Pityrosporum spec. and consists of 40 to 90 parts by weight of a customary base medium and one or more selectors selected from the group consisting of glycerol monostearate, Tween 80, chloramphenicol and gentamycin.

10. The method according to claim 1, wherein the selective medium is for testing yeasts and consists of 30 to 50 parts by weight of a customary base medium and one or more selectors selected from the group consisting of bismuth sulphite and neomycin.

11. The method according to claim 1, wherein the yeast is from the genus Candida.

12. The method according to claim 1, wherein the selective medium is for testing molds or for dermatophytes and consists of 20 to 75 parts by weight of a customary medium and NaCl as the selector.

13. The method according to claim 1, wherein the selective medium is for testing enterococci and consists of 30 to 60 parts by weight of a customary base medium and one or more selectors selected from the group consisting of sodium citrate, sodium azide, thallium acetate and 2,3,5-triphenyltetrazole.

14. The method according to claim 1, wherein the selective medium is for testing coliform organisms and consists of 30 to 60 parts by weight of a customary base medium and one or more selectors selected from the group consisting of NaCl, lactose, basic fuchsin and Na.sub.2 SO.sub.3.

15. The method according to claim 14, wherein the coliform organism is Escherchia coli.

16. The method according to claim 1, wherein the selective medium is for testing Enterobacterieaceae and consists of 30 to 60 parts by weight of a customary base medium and one or more selectors selected from the group consisting of sodium

citrate, Na.sub.2 S.sub.2 O.sub.3, sodium deoxycholate, ammonium icon (III) citrate and neutral red.

17. The method according to claim 1, wherein the dermatological disease is

atopic eczema

psoriasis

acne

seborrheoic dermatitis

cellulitis caused by bacteria

dermatomycoses

superinfections of the skin with pathogenic and/or apathogenic Gram-positive and/or Gram-negative microorganisms.

WEST

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L21: Entry 1 of 1 File: USPT Feb 16, 1999

clm 9

Am 9 Tangent Medium

DOCUMENT-IDENTIFIER: US 5871952 A
TITLE: Process for selection of Oxygen-tolerant algal mutants that produce H.sub.2

Detailed Description Text (11):
For H.sub.2 -production selection, anaerobically-treated cells (without addition of an O.sub.2 scrubbing system) were added to a selective medium containing different concentrations of metronidazole and 1 mM sodium azide (.sup.8). The azide inhibits endogenous catalase activity. All procedures were done under sterile conditions. The selection medium was also made anaerobic by argon bubbling before introduction of the cells. Oxygen was then added to the medium to achieve final concentrations of O.sub.2 in the gas phase ranging from 0-10% or higher, as required. The final cell suspension was exposed to light of controlled intensity (Fiber-Lite High Intensity Illuminator, model 170-D Dolan-Jenner Industries, Inc.) for 20 minutes. The cells were pelleted out using a clinical centrifuge, washed once with phosphate buffer, pH 7.0, and then once with resuspension buffer (5 mM potassium phosphate buffer containing 1 mM CaCl.sub.2 and 1 mM Mg.sub.2 SO.sub.4). Undiluted and sequential dilutions of each sample were plated on minimal medium and incubated in a growth chamber under low light levels. Survival rates were determined by counting the number of colonies detected on each plate following the treatment, and estimating the percentage of survivors with respect to the number of cells at the beginning of the MNZ treatment.

WEST

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L6: Entry 12 of 176 File: PGPB Oct 4, 2001

Notes

DOCUMENT-IDENTIFIER: US 20010026924 A1
TITLE: Noninvasive detection of colorectal cancer and other gastrointestinal pathology

CLAIMS:

14. The transport medium of claim 13, wherein the bacteriocidal agent is selected from the group consisting of thimerosal, antibiotics and sodium azide.

WEST

Generate Collection | Print

L6: Entry 131 of 176 File: USPT Sep 8, 1981

DOCUMENT-IDENTIFIER: US 4288543 A
TITLE: Method and apparatus for identifying microorganisms

CLAIMS:

1. A method of identifying a strain of microorganism in a liquid sample comprising the steps of dividing the sample into at least 18 sub-samples, inoculating each of the sub-samples with a growth-inhibiting agent whose reaction with the sub-samples has characteristics capable of identifying the strain of microorganism in said sample, the identity and/or quantity of the growth-inhibiting agent inoculated into each of said sub-samples being different, incubating the sub-samples to develop potentially significant differences in the growth of microorganism in each of them, reading a measure of the growth of the microorganism in each sub-sample, and comparing the growth readings with a bank of microorganism-identifying data relative to said growth-inhibiting agents by quadratic discriminant function analysis, whereby the strain of microorganism in the sample is identified, said growth-inhibiting agents being selected from the group consisting of acriflavine, 9-amino-acridine, auramine O, brilliant green, cetrimide, cobalt chloride, cupric chloride, cycloserine, 3,5-dibromosalicylic acid, dodecylamine hydrochloride, 5-fluorouracil, floxuridine, malachite green, methylene blue, omadine disulfide, sodium omadine, sodium azide, thallous acetate, 2',3',4'-trihydroxyacetophenone, bacitracin, carbenicillin, cephalothin, colistin, kanamycin, methenamine mandelate, nalidixic acid, nitrofurantoin, novobiocin, polymyxin B and tetracycline.

4. A method of claim 3, wherein the concentrations of growth-inhibiting agents in the inoculated sub-samples are approximately as follows: acriflavine (5 or 20 mcg./ml), 9-aminoacridine (6.7 mcg./ml.), auramine 0 (107 mcg./ml.), brilliant green (1,2 or 3.3 mcg./ml.), cetrimide (80 mcg./ml.), cobalt chloride (250 mcg./ml.), cupric chloride (250 mcg./ml.), cycloserine (80 or 160 mcg./ml.), 3,5-dibromosalicylic acid (500 mcg./ml.), dodecylamine hydrochloride (12.5 or 50 mcg./ml.), 5-fluorouracil (5.3 mcg./ml.), floxuridine (6 or 24 mcg./ml.), malachite green (2 mcg./ml.), methylene blue (170 mcg./ml.), omadine disulfide (3.7 mcg./ml.), sodium omadine (5 mcg./ml.), sodium azide (50 mcg./ml.), thallous acetate (100 mcg./ml.), 2',3',4'-trihydroxy-acetophenone (250 mcg./ml.), bacitracin (12 units/ml.), carbenicillin (33 mcg./ml.), cephalothin (10 mcg./ml.), colistin (8.7 mcg./ml.), kanamycin (3.3 mcg./ml.), methenamine mandelate (667 mcg./ml.), nalidixic acid (4 mcg./ml.), nitrofurantoin (10 mcg./ml.), novobiocin (20 mcg./ml.), polymyxin B (33 mcg./ml.), tetracycline (0.33 mcg./ml.).

WEST

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L6: Entry 5 of 176 File: PGPB Jun 13, 2002

DOCUMENT-IDENTIFIER: US 20020071875 A1
TITLE: Azide method and composition for controlling deleterious organisms

CLAIMS:

14. A composition for controlling a population of a deleterious organism comprising: an aqueous liquid medium; an azide salt selected from the group consisting of sodium azide and potassium azide; and an azide stabilizer, wherein the composition exhibits a pH greater than about 8.7.

16. A method of controlling a population of a deleterious organism, in soil, comprising the step of: applying to a soil a composition comprising an azide, a liquid medium, and an azide stabilizer, wherein the amount of azide in the soil is effective for controlling a population of a deleterious organism therein.

26. A method for delivering a pesticidal composition comprising: applying to soil a pesticidal composition exhibiting a pH value greater than about 8.7, wherein the pesticidal composition comprises an aqueous liquid medium, an azide selected from the group consisting of potassium azide and sodium azide, and a component selected from the group consisting of detergents, pH buffering agents, amines, amino acids, oligopeptides, polypeptides.

WEST

☐ Generate Collection | Print

L30: Entry 17 of 28 File: USPT Apr 15, 1997

clm 1-2

DOCUMENT-IDENTIFIER: US 5620865 A
TITLE: Medium for detecting Enterococci in a sample

Brief Summary Text (9):
Enterococcus density is a predictor of public health risks associated with contaminated recreation waters. There are two accepted methods for the analysis of Enterococcus density in water samples, the multiple-tube for most probable number technique (MPN) and the membrane filter technique (MF) (Greenberg et al., Standard methods for the evaluation of water and wastewater Eaton, A.D. (ed.) 18th ed. American Public Health Association (1992); and Mooney, K. et al., Testing the waters: a national perspective on beach closings Natural Resources Defense Council. (1992)). The results based on the multiple-tube technique may not be available for 72 hours, and the results of the membrane filter technique may not be available for 48 hours. The "MPN procedure" involves a 24 to 48 hour presumptive test in a series of azide dextrose broth followed by a 48 hour confirmation test using selective Enterococcus agar and 6.5% NaCl brain-heart infusion broth. The membrane filter technique involves the membrane filtration of water samples followed by incubation of a pre-filtered sterile membrane on Enterococcus selective media. The media of choice are either mE agar followed by an EIA substrate test, or menterococcus agar. Such methods may be tedious, labor intensive and time consuming. This may lead to delays in public notification and therefore increase public health risks.

WEST

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L30: Entry 4 of 28 File: USPT Mar 6, 2001

DOCUMENT-IDENTIFIER: US 6197574 B1
TITLE: Bacterium detector

Detailed Description Text (62):
With regard to a medium for Staphylococcus aureus, it is possible to use mannitol-salt (modified) medium, Baird-Parker medium, tellurite-glycine medium, phenylethanol-azide medium, chocolate-agar medium, blood-agar medium, heart infusion agar medium, etc. in the present invention and, in view of the adaptability with the later-mentioned antibiotic substance for selecting Staphylococcus aureus, the use of a mannitol-salt (modified) medium is preferred.

WEST

☐ Generate Collection Print

L29: Entry 12 of 70 File: USPT Mar 12, 2002

DOCUMENT-IDENTIFIER: US 6355449 B1
TITLE: Method and medium for detecting vancomycin-resistant enterococcus

Clm 1

CLAIMS:

1. A medium for detecting vancomycin-resistant Enterococci in a sample form a rectal swab, peri-rectal swab, or stool sample comprising:

vancomycin in an amount sufficient to suppress the growth of vancomycin sensitive Enterococci;

a first nutrient indicator which is a substrate for a first bacterial enzyme and provides a first detectable signal when cleaved by the first bacterial enzyme wherein the first nutrient indicator is a substrate for--.beta.-glucosidase;

a second nutrient indicator which is a substrate for a second bacterial enzyme and provides an intermediate molecule when cleaved by the second bacterial enzyme, and the intermediate molecule provides the second detectable signal upon reacting with a developing agent, wherein the second detectable signal is distinct from the first detectable signal wherein the second nutrient indicator is a substrate for pyrrolidonyl arylamidase;

an effective amount of one or more selective agents active to prevent or inhibit the growth of microorganisms other than Enterococci when a sample from a rectal swab, peri-rectal swab, or stool sample is introduced into the medium wherein the one or more selective agents are selected form the group consisting of: amikacin sulfate, polymyxin B, bacitracin, clindamycin, cefotaxime, amphotericin B, sodium azide, thallium acetate, nalixidic acid, enoxacin, cinoxacin, ofloxacin, norfloxacin, cefotaxime, gentamycin, neomycin, polymyxin B, colistin, and bile salts.

WEST

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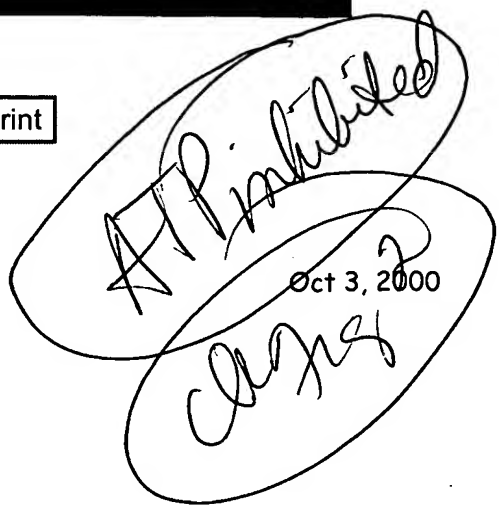

L19: Entry 4 of 11 File: USPT Oct 3, 2000

DOCUMENT-IDENTIFIER: US 6127167 A
TITLE: Method of controlling proliferation of aerobe

Abstract Text (1):
Disclosed herein is a method of controlling the proliferation of an aerobe, in which in the continuous culture of the aerobe by supplying a substrate and oxygen to a culture tank of the aerobe to cause the aerobe to decompose the substrate, the proliferation of the aerobe is inhibited while retaining the substrate-decomposing activity inherent in the aerobe. An oxidation-reduction substance which is reduced by electrons donated by an electron transport system of the aerobe and oxidized by oxygen supplied to the culture tank is caused to coexist with the aerobe in the culture tank.

Brief Summary Text (14):
In order to achieve the above object, in an aspect of the present invention, there is thus provided a method of controlling the proliferation of an aerobe, in which in the continuous culture of the aerobe by supplying a substrate and oxygen to a culture tank of the aerobe to cause the aerobe to decompose the substrate, the proliferation of the aerobe is inhibited while retaining the substrate-decomposing activity inherent in the aerobe, wherein an oxidation-reduction substance which is reduced by electrons donated by an electron transport system of the aerobe and oxidized by oxygen supplied to the culture tank is caused to coexist with the aerobe in the culture tank.

Detailed Description Text (12):
According to the method of the present invention, the oxidation-reduction substance coexists with the aerobe in the culture tank. Therefore, in the electron transport system, the electrons formed by the charge separation at the coupling site 13 or the coupling site 17 are donated to the oxidation-reduction substance. As a result, the concentration gradient of proton formed in the above-described manner becomes insufficient, and so the synthesis of ATP in the electron transport system is inhibited. ATP synthesized in the electron transport system accounts for about 70% of ATP formed in the whole aerobic respiration system. Therefore, if the synthesis of ATP in the electron transport system is inhibited, ATP is lacking, and so the proliferation is inhibited.

Detailed Description Text (15):
The oxygen used may be supplied in the form of a mixture with other one or more gases. Air is generally supplied as a gas containing oxygen. Preferable examples of the aerobe include aerobic bacteria, facultative anaerobic bacteria, actinomycete, yeast, mold and basidiomycete. These aerobes may be used either singly or in any combination thereof.

Detailed Description Text (30):

In this time, thionine, which is an oxidation-reaction substance, exists in the form of the solution together with the aerobe in the aeration tank 2. Therefore, electrons are donated to thionine at the coupling site 13 in the electron transport system illustrated in FIG. 2, upon the aerobic respiration of the aerobe. As a result, the electrons are not transferred beyond the coupling site 13 in the electron transport system illustrated in FIG. 2, whereby the synthesis of ATP is inhibited, and so the proliferation of the aerobe is inhibited.

CLAIMS:

7. The method of claim 1, 2, or 3 wherein the aerobes comprise facultative anaerobic microorganisms.